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THE EFFECT OF DIET UPON THE BLOOD THIONEINE OF THE ALBINO RAT[1]

VAN R. POTTER AND KURT W. FRANKE

Department of Experiment Station Chemistry, South Dakota State College, Brookings

TWO FIGURES

(Received for publication June 21, 1934)

While assaying grain samples for the new plant toxicant described by Franke ('34), chemical studies on blood from rats on a variety of dietary regimes were made. In this paper there will be reported the findings for thioneine and uric acid. Glutathione was determined on a few samples of blood to determine whether or not the thioneine variations correlated with glutathione concentrations.

EXPERIMENTAL

The rats were all of Wistar Institute origin and, with the exceptions of the rats on the potato diet, all were in good physical condition at the time of autopsy. The majority of the animals had served as controls in various experiments and had been kept in individual cages. The rats on the potato diet showed poor growth and certain abnormalities which have been described by Kon ('31).

Diets. The barley, corn, wheat, oats, and potato diets had the composition shown in table 1.

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[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 4 from the Department of Experiment Station Chemistry.

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TABLE 1

	CORN DIET	WHEAT DIET	BARLEY DIET	OATS DIET	POTATO[1] DIET
Ground grain	82.0	82.0	82.0	82.0	82.0
Casein	10.0	10.0	10.0	15.0	10.0
McCollum's salt mixture no. 185	1.0	1.0	1.0	1.0	4.0
Cod liver oil	2.0	2.0	2.0	1.0	2.0
Northwestern dried yeast	2.0	2.0	2.0	1.0	2.0
Lard	3.0	3.0	3.0	...	...

[1] The potatoes used in this diet were sliced, dried, and ground to a fine powder.

The casein-sucrose low-thioneine diet had the following composition:

	Per cent		Per cent
Sucrose	45	Butter	10
Commercial casein	20	McCollum's salt	3
Northwestern dried yeast	10	Cod liver oil	2
Corn starch	10		

This diet produces excellent growth.

The stock diet consisted of:

	Per cent		Per cent
Wheat	25.0	Alfalfa meal	5.0
Corn	25.0	Ground peas	5.0
Rolled oats	20.0	Cod liver oil	3.0
Fish meal	10.0	NaCl	1.0
Wheat germ	5.0	$CaCO_3$	1.0

ANALYTICAL METHODS

Blood was obtained from the abdominal aorta by the method of Swanson and Smith ('32). Two mg. of $K_2C_2O_4$ per cubic centimeter of blood were used to prevent clotting. The tungstomolybdic acid method (Benedict, '31) of preparing protein-free filtrates has been used throughout this study. The determination of thioneine and uric acid is essentially a combination of Benedict and Behre's procedure ('31) with the reagents recently described by Folin ('30).[2]

Folin's reagents have been previously used by Salt ('31) for the determination of thioneine. Salt also used a uric acid standard for his comparisons. Folin's uric acid reagent is preferred, because it is relatively free from phenol reagent. Furthermore, the use of the cyanide-urea reagent, as described below, eliminates turbidities and the solutions may be compared when cold. As for color values, Folin's uric acid reagent gives colors which are practically identical to the color produced by Benedict's reagent with equal amounts of pure solutions of glutathione or uric acid. Benedict's reagent gives more color with thioneine than does the Folin reagent, but it was thought advisable to use the same reagent for both determinations. Pyrex test tubes 150 × 16 mm. without lip (Board of Health tubes) have been used. These tubes fit the centrifuge cups ordinarily used for 15 cc. conical centrifuge tubes.

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[2] This investigation was completed prior to Folin's later article ('33) which does not, however, affect the validity of the results presented here.

Method for determining uric acid. To 10 cc. of protein-free filtrate in a test tube add 2.5 cc. of acid lithium chloride (Benedict and Behre, '31). Mix well, and add 2.5 cc. of silver nitrate solution (Benedict and Behre, '31). Shake well, stopper, and centrifuge thoroughly. Carefully pour off top liquid into a clean test tube, and invert the tube with the residue on a pad of filter paper for a few minutes. This residue will be used for the thioneine determination. Pipette 5 cc. of the thioneine-free filtrate into a clean test tube, add 5 cc. of the cyanide-urea (with excess calcium) (Folin, '30) followed by 1 cc. of uric acid reagent (Folin, '30), noting the time. Both reagents should be added from burettes. Invert twice, and stopper with cork. At the end of exactly 4 mintues, immerse in boiling water for exactly 2 minutes. Remove to a rapidly circulating cold water bath for 4 or 5 minutes, centrifuge thoroughly for 1 or 2 minutes, and compare with a suitable standard, which is set at 20 mm. in the left cup of the colorimeter; $20/R \times 0.004 \times$ cc. dilute standard $\times 300 =$ mg. uric acid per 100 cc. The accurate timing during the color development is no hardship once a schedule has been worked out. In this laboratory, cyanide-urea is added to all the tubes, after which the color reagent is added at 10-second intervals to twelve tubes. The color reagent is added to a second group of twelve tubes while the first group is being cooled. Twenty-four tubes may be run in two groups of twelve each, with but four standards in the series. Turbidities never occur.

Method of determining thioneine (in terms of uric acid). Dissolve the silver chloride residue from the indirect uric acid determination (see above) in 5 cc. of cyanide-urea, add 5 cc. of water, followed by 1 cc. of the uric acid reagent and proceed exactly as in the determination of uric acid above; $20/R \times 0.004 \times$ cc. dilute standard $\times 100 =$ UA-mg. of thioneine (thioneine in terms of mg. uric acid) per 100 cc. (whole blood). All thioneine values are reported as UA-mg. of thioneine.

Standards for thioneine and uric acid. The stock standard is prepared according to Folin ('30). For the determinations, four standards are prepared, using 0.5 cc., 1 cc., 2 cc., and 3 cc. of the dilute standard and water sufficient to make 5 cc. The standards are run parallel to the blood samples and the four described suffice for both the thioneine and the uric acid determinations.

Method for determining glutathione. The method of Benedict and Gottschall ('33) was used,[3] with the exception of the uric acid reagent for the color development, which was that of Folin ('30). The color produced by Folin's uric acid reagent with glutathione in the conditions of this determination are virtually identical with the colors produced by Benedict's reagent, and the former has the advantage of not giving tests for phenols. Volumes of filtrate and reagents were doubled throughout to facilitate color comparison. The authors are aware of the fact that the method has not been previously used on rat blood. Data presented in this paper show quite conclusively that the determination is not affected by thioneine variations in excess of 1000 per cent.

DISCUSSION OF RESULTS

The results of the thioneine analyses on blood from 134 rats on various diets are shown in figure 1. The levels as observed according to diet were as follows: wheat, 0.43 ± 0.18 UA-mg.;

[3] Glutathione from Eastman Kodak Company was used for standards.

corn, 0.93 ± 0.22 UA-mg.; two samples of oats had levels of 1.22 and 0.59 UA-mg., respectively; stock diet yielded an average of 1.17 ± 0.20 UA-mg. A careful study of the data failed to reveal significant differences due to sex or age.

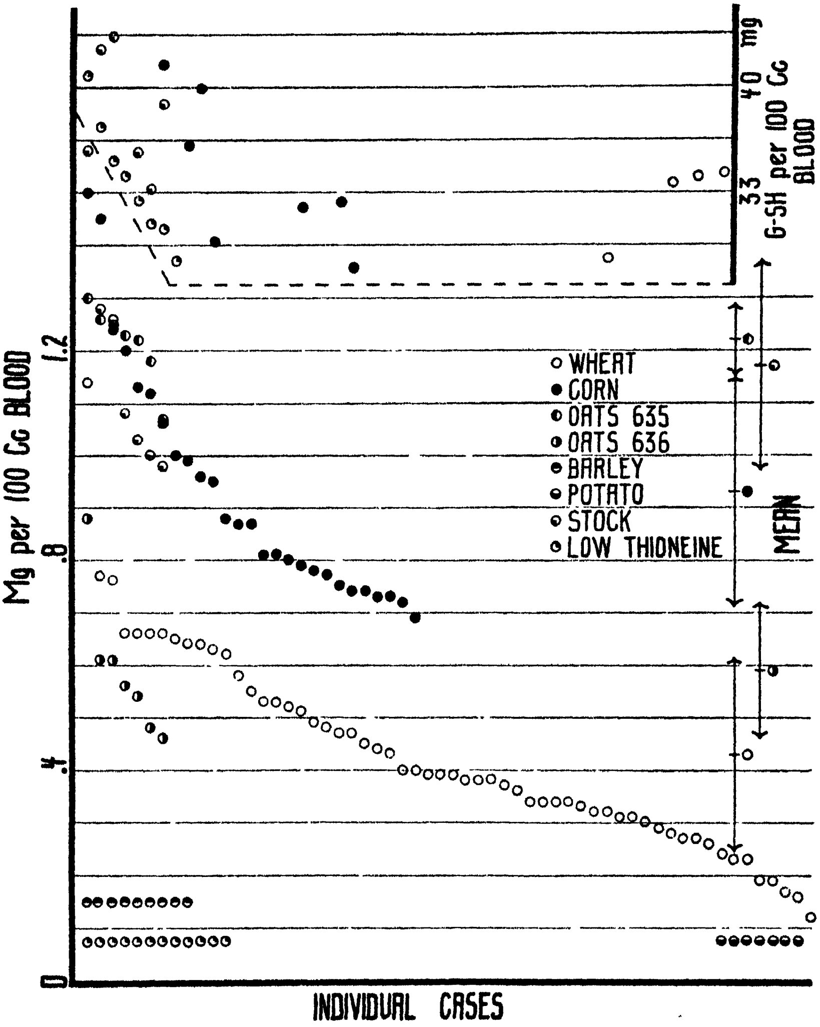

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Fig. 1 Results of consecutive blood thioneine determinations on 134 white rats on eight different diets, with mean values and standard deviations for each diet. Glutathione values on twenty-four consecutive cases are given directly above corresponding thioneine values. Thioneine values are expressed in UA-mg. (thioneine in terms of uric acid) and plotted in order of decreasing values.

Figure 1 shows that among the common cereal grains, barley is extremely low in the precursor for thioneine, while corn is rich in the precursor. The wheat mean indicates that wheat is also relatively low in the precursor. The figures presented for the two samples of oats are very interesting, since they show that marked variations can occur between different samples of the same kind of grain. The figures on the two samples of oats were obtained on two separate occasions and rats on both samples were taken each time. The analytical findings in figure 1 seem to prove that blood thioneine is entirely exogenous in character. Furthermore, blood from young rats at weaning time has been found to be practically free from thioneine. Eagles and Vars ('26) found that pigs which received corn diets had high thioneine levels and pigs on garbage or casein diets had thioneine values so low as to be undetectable in some cases. The glutathione values recorded in figure 1 show that the thioneine variations are not accompanied by variations in glutathione.

Benedict, Newton, and Behre ('26) have reported thioneine levels in various pathological bloods. They found that diabetics showed a tendency toward higher than normal levels, while nephritics had normal or low values. Their work was confirmed by Salt ('31). The work presented in this paper, showing the marked effect of diet upon blood thioneine, causes the clinical significance of blood thioneine findings to be questionable. It seems entirely possible that the dietary regimes of diabetic and nephritic patients might be responsible for their blood thioneine levels.

Eagles and Vars ('28) suggested thiolhistidine as a precursor for thioneine. This, however, seems highly improbable from the work of Harington,[4] who is of the opinion "that thiolhistidine either does not occur at all, or only occurs in the most minute quantities in the cereal protein." The thioneine levels observed in the rats on the low-thioneine diet are interesting, because they seem to rule out a number of possible precursors. Casein is comparatively rich in methio-

[4] Personal communication from C. R. Harington.

nine (Pirie, '32) and yeast has been shown to contain considerable glutathione (Tunnicliffe, '25). Since the low-thioneine diet contains 20 and 10 per cent, respectively, of casein and yeast, it seems quite likely that neither methionine nor glutathione is the precursor for thioneine. The fact that the diet was entirely adequate indicates that it was at least not deficient in histidine or cystine.

Figure 2 presents the uric acid values which were observed in all the blood samples used in figure 1. A survey of the data has failed to reveal significant differences in uric acid values according to diet, age or sex. The mean for the group of 127 rats (potato rats excluded) has been calculated and found to be 3.38 mg./100 cc. with a standard deviation of ± 1.55 mg.

There have been very few publications regarding the blood chemistry of the albino rat. Folin and Morris ('13) analyzed the pooled blood of six normal rats and found 2 mg. of uric acid per 100 cc. of blood. Anderson, Honeywell, Santy, and Pedersen ('30) found an average of 1.86 mg. of uric acid per 100 cc. of blood in seven rats. Turner ('20) found from 2.6 to 3.1 mg. of uric acid per 100 cc. in the blood of twelve normal stock rats and from 2.5 to 3.8 mg. in sixteen control rats. With the exception of the report by Folin and Morris, the figures were based upon tungstic acid filtrates, using Benedict's direct method for uric acid.

Up to this time, thioneine estimations involving the uric acid reagent have utilized the silver residue remaining after the indirect removal of uric acid by means of the silver lactate —acid sodium chloride treatment of blood filtrates (Behre and Benedict, '29; Benedict, Newton, and Behre, '26; Rockwood, Turner, and Pfiiffner, '29; Salt, '31). The author's modification for the thioneine determination involves the use of the silver residue which is obtained in Benedict and Behre's ('31) new method for determining uric acid, and leaves little to be desired from the standpoint of simplicity. Behre ('32) has questioned all methods (Benedict, Newton, and Behre, '26; Rockwood, Turner and Pfiffner, '29; Salt, '31) involving cyanide as the sole alkali. She states that when sodium hydroxide was employed as the alkali, and a minimum of cyanide was used, lower thioneine figures were obtained (Behre and Benedict, '29), but added thioneine was completely recovered.

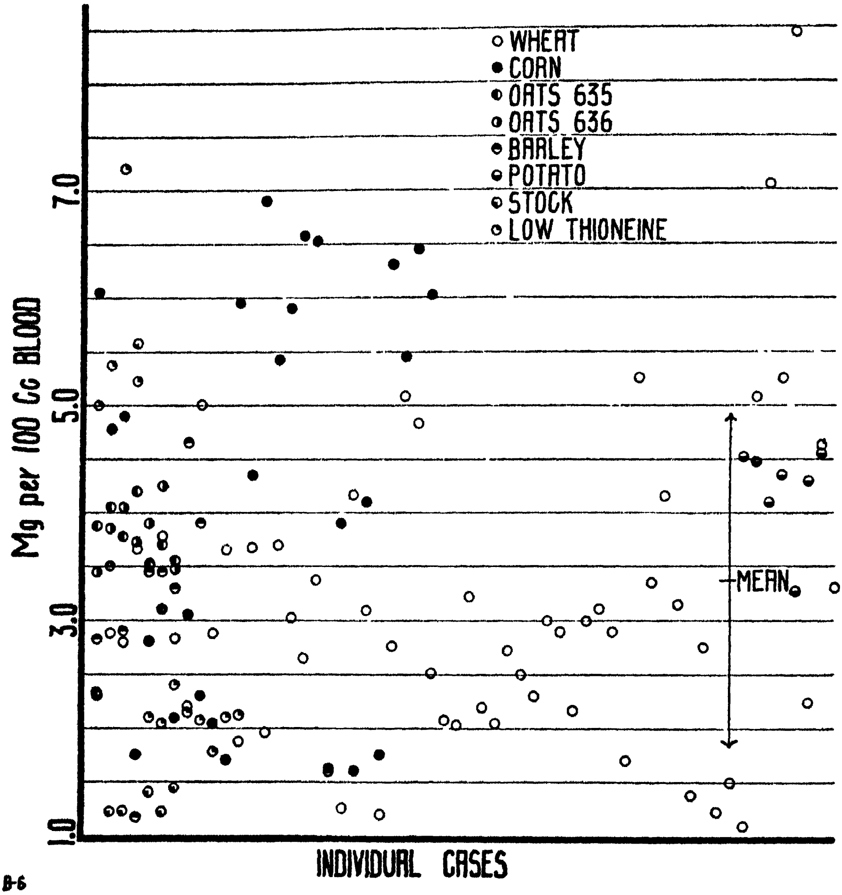

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Fig. 2 Results of uric acid determinations on 134 consecutive samples of rat blood in eight different diets, plotted in same order as samples in figure 1. The mean value and standard deviation of the entire group, excluding potato rats, is given.

The authors' method for thioneine is probably in positive error by about 0.075 UA-mg. Experiments in this laboratory have shown that aqueous solutions containing glutathione equivalent to 21, 35, or 49 mg. per 100 cc. of blood give almost identical colors when analyzed as for thioneine, the color being approximately equal to that given by a solution of uric acid equivalent to 0.075 mg. per 100 cc. The glutathione values for the rats on the low thioneine diet (fig. 1) could therefore account for the thioneine values observed, and the blood of this group of animals may actually have been thioneine-free.

When silver chloride is precipitated in an aqueous solution of glutathione, according to the procedure for thioneine, the glutathione is not quantitatively carried down with the precipitate. Experiments on the inhibitory effect of glutathione upon the uric acid determination, to be published elsewhere, indicate that the indirect uric acid determination may still be affected by glutathione. The authors were unable to make a thorough investigation of the accuracy of the thioneine method by recovery experiments or by determining the effect of possible interfering substances. From a theoretical standpoint, however, the method should be as accurate as that described by Salt ('31) and has the advantages of being much easier to carry out. Thioneine was expressed in terms of uric acid, because it was felt that the extra labor involved would add very little to the significance of the figures presented, which are relatively correct and show clearly the effect of the various cereal grains on blood thioneine. It is hoped that these figures may prove of benefit in the search for a precursor for blood thioneine.

SUMMARY

1. Blood thioneine is shown to be entirely exogenous and the levels observed in 134 rats on eight different diets are presented.
2. No precursor for thioneine is suggested. The precursor is probably not methionine, glutathione, or thiohistidine.
3. The average of the blood uric acid values in 127 rats is presented.
4. Modified methods for the determination of uric acid and thioneine are presented.

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LIVER GLYCOGEN FROM DERIVATIVES OF GLUCOSE

W. T. SALTER, P. D. ROBB AND F. H. SCHARLES
Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Boston

FOUR FIGURES

(Received for publication May 28, 1934)

Many investigators have studied the ability of the liver to store various foodstuffs in the form of glycogen. The conversion of naturally occurring hexoses (like galactose and fructose) into liver glycogen has been well described (Cori, '26; and Cori and Cori, '27). This type of chemical transformation has even been suggested as a test of liver function (Banks, Sprague and Snell, '33). Nevertheless, the versatility of this chemical synthetic capacity of the hepatic cells has not as yet been completely described in its qualitative aspects.

Drury and Salter ('34) administered a series of glucose derivatives to rabbits whose livers had been removed. They found that substitution of even a single chemical group in the hexose molecule usually rendered the sugar useless for such animals. When given to normal animals (mice), however, many of these carbohydrates were stored as liver glycogen. This paper records liver glycogen formation by six carbohydrates for which data in the extant literature are scanty or lacking. For purposes of comparative standards, this paper also describes the glycogen content of livers in fasting mice when a single feeding of glucose is administered.

The dose of glucose used is varied in successive experiments, so that a considerable range of nutrition is covered. Glycogen storage can be demonstrated in the liver soon after the sugar is administered; but as fasting continues, the glycogen disappears. The rate of absorption, and the height of liver glycogen depend upon the amount of glucose administered. Four sets of data are presented, representing four levels of carbohydrate storage. It is hoped that the data may serve as standard curves of reference for the study of carbohydrate turnover in mice.

EXPERIMENTAL PROCEDURE

Young, mature mice of both sexes were used for these experiments. The stock was fed bread, hemp and millet seed, crackers, cabbage, milk, and cod liver oil. Prior to the experiment, they were fasted for 48 hours and then fed through stomach tube a standard amount of sugar in solution. (The selection of standard amounts is described below.) The use of a small glass tube, suitably molded, attached to a tuberculin syringe, permits accurate delivery of liquid. The animals were allowed water ad libitum. They were kept in individual fruit jars in a thermostat controlled animal room at 70° F. At various intervals after feeding, groups of animals were sacrificed.

The animals were stunned, carotid arteries cut, and allowed to bleed for half a minute. The liver was then removed as rapidly as possible and dropped into a tared tube containing 1.5 cc. of 60 per cent warm potassium hydroxide. Each tube was stoppered with a glass bulb, to the undersurface of which a stirring rod was attached. The tissue was speedily disintegrated. The tube was then cooled and weighed again. Ligatures were next placed about the esophagus and rectum and the gastro-intestinal tract removed to a beaker. The contents of the gut were then washed out with several successive portions of hot water, and collected in a volumetric flask. In this manner, unabsorbed sugar could be determined.

CHEMICAL METHODS

The chemical procedures followed were essentially those described by Cori ('25 a). Glycogen was precipitated after

3 hours' digestion (in aqueous potassium hydroxide at 100° C.) by adding water and alcohol. Two methods were employed in the final estimation of the sugar produced by acid-hydrolysis of the glycogen. The colorimetric method of Folin ('29) proved as accurate as the iodometric titration method of Somogyi, modified from Shaffer-Hartman (Shaffer and Somogyi, '33), but the latter conveniently obviated preliminary determinations of glucose for approximate concentration.

The washings from the gut were deproteinized with colloidal iron as described by Cori ('25 b). The filtrates were then analyzed for reducing substance. Control, fasted animals yielded negligible values by this procedure.

The accuracy of the experimental procedure was controlled by several parallel experimental manipulations. Delivery from the syringe was much more constant than the biological reaction of the animals. The intestines and stomachs of fasted animals yielded negligible reducing values. Livers of 48-hour fasted mice contained (apparently) 0.07 per cent of glycogen which was allowed as a blank. The accuracy of the glycogen determination itself was tested by preparing glycogen from mouse livers by the method of Pflüger, modified by Cori (personal communication), and then analyzing known amounts, both separately and when added to fasting liver tissue. The recovery of added glycogen was 95 ± 3 per cent of theoretical.

INGESTION LEVEL

All the results here reported concern the fate of carbohydrate solutions delivered into the stomach. The maximum amount which can be administered in this fashion depends, first, upon the capacity of the stomach and, secondly, upon the tolerance of the gut to irritating hypertonic solutions. Mice weighing about 25 gm. were selected, and solutions so made up that about $\frac{1}{2}$ cc. of solution was given. The actual amount of sugar to be administered was determined by the weight of each animal. Regurgitation and diarrhea were carefully looked for, and such animals excluded. Even in the absence of diarrhea, the intestine may be markedly distended by fluid soon after feeding a large amount of sugar.

It was found that the gut tolerates more of glucose than of most other sugars. Accordingly, glucose feeding was conducted at several distinct levels (maximal, two intermediate, and low) so that the effect of other sugars could be compared at similar ingestion levels.

SPECIAL CARBOHYDRATES STUDIED

Of the special carbohydrate substances used, five were hexose derivatives. These included sorbitol, glucosamine, gluconic acid, and the two glucosides of methyl and ethyl alcohol, respectively. The chemical configuration of these or similar compounds is given by Herring, Irvine and Macleod ('24), as well as by Armstrong ('24). The sorbitol was purchased from Pfanstiehl Company. The gluconate (calcium salt) was generously contributed by Sandoz Chemical Company. The other hexose derivatives were prepared in this laboratory: e.g., α-ethyl d-glucoside.

In addition, a glycerol derivative, propylene glycol (propane-diol 1, 2) was also tested. This propylene glycol was obtained from the National Carbon Company through the courtesy of Dr. Reid Hunt.

EXPERIMENTAL DATA FOR GLUCOSE

Table 1 shows both the change in liver glycogen concentration and in the fraction of the glucose fed which was recovered as glycogen. Two sets of data are given for the maximal dosage curve, one obtained during the winter months and the other in the summer. No significant difference is evident. For the latter curve, pedigreed inbred black mice were employed as well as stock white mice. They showed no obvious difference in behavior.

The maximal level is presented in duplicate. This last level represents the maximum amount of glucose which the gastrointestinal tract would tolerate consistently.

TABLE 1

Recovery of liver glycogen in mice after feeding glucose in various doses

		TIME AFTER FEEDING	NUMBER OF MICE			AVERAGE GLYCOGEN CONTENT OF LIVER	AVERAGE FRACTION OF INGESTED GLUCOSE AS LIVER GLYCOGEN
			Used	Discarded	Died		
		Hours				*Per cent*	*Per cent*
Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse		4¼	6	1		0.09	4.7
		6	3			0.12	5.2
		8	6			0.13	6.4
		13½	8	1		0.14	6.9
Molecular glucose—equivalent: 0.2 gm. per 100 gm. of mouse		4	9			0.85	22.3
		6½	7			0.75	17.8
		12½	6			0.48	11.7
		24½	7			0.08	2.3
Molecular glucose—equivalent: 0.5 gm. per 100 gm. of mouse		1	5			1.20	10.9
		2	5			2.00	20.3
		3	4	1		2.49	22.3
		4	8			1.64	17.0
		6	16	1		1.11	11.8
		10	12	1		0.69	6.8
		15	8	2		1.16	11.5
		20	9	2		0.51	4.6
Molecular glucose—equivalent: 0.7 gm. per 100 gm. of mouse	Summer curve	4¼	12			2.77	17.7
		4½	6			2.21	16.4
		4¾	9			2.40	14.4
		6	4			4.39	26.5
		10	5			2.70	19.0
		18	7			0.96	5.9
		24	5			0.29	1.5
	Winter curve	¼	2			0.83	7.8
		½	3	1		0.85	7.1
		1	14			1.17	10.8
		2	10			1.93	14.6
		3	12			2.85	20.3
		4	13			2.49	19.1
		5	11	1		3.00	24.2
		6	7			2.71	19.8
		7	5			2.42	19.9
		11	4			2.44	18.1
		13	4			2.13	16.2
		15	4			2.03	12.4
		16	4			1.78	11.3
		17	4			1.44	8.6
		20	6	2		1.30	9.7
		24	7	2	2	0.85	4.7
		30	5			0.47	2.8
		40	3		3	0.88	5.5
		48	4	1		0.14	0.8
		Blank	10			0.07	

The rate of intestinal absorption for the highest level is given in table 2. These data for mice, obtained by a method similar to Cori's ('26) procedure in rats, show a somewhat different trend in relation to time from the results obtained on rats.

Figure 1 shows that the postprandial content of glycogen in the liver becomes progressively higher as increasing amounts of glucose are administered. The limiting value is fixed, not by the liver, but by gastro-intestinal tolerance to a single large feeding. That is, up to the point where diarrhea interferes, liver glycogen concentration is greater when larger amounts of glucose are fed.

TABLE 2

Intestinal absorption in mice after feeding glucose and derivatives of glucose

	TIME AFTER FEEDING	NUMBER OF MICE	AVERAGE ABSORPTION PERCENTAGE OF CARBOHYDRATE FED
	Hours		*Per cent*
Glucose	¼	3	24.6
	½	4	59.2
	1	6	78.8
	2	2	91.4
	3	4	95.7
	4	5	96.5
	5	3	96.9
Propylene glycol	½	3	77.8
	1	3	61.6
	1½	3	74.7
	2	6	81.8
	3	8	84.7
	5	3	93.7
	7	6	93.6
Glucosamine	2	3	45.4
	5	4	80.6
Ethyl glucoside	2	3	46.1
	5	1	58.2

By way of contrast, the fraction of fed glucose which the liver stores remains nearly the same despite wide variation in the actual amount fed. This is not true when a very small amount of sugar is fed; but applies over a considerable range of dosage. Figure 2 shows that the total glycogen stored in the previously fasted liver is roughly proportional to the amount of glucose fed, provided that the dose of sugar is a significant portion of the maximal dose which is tolerated.

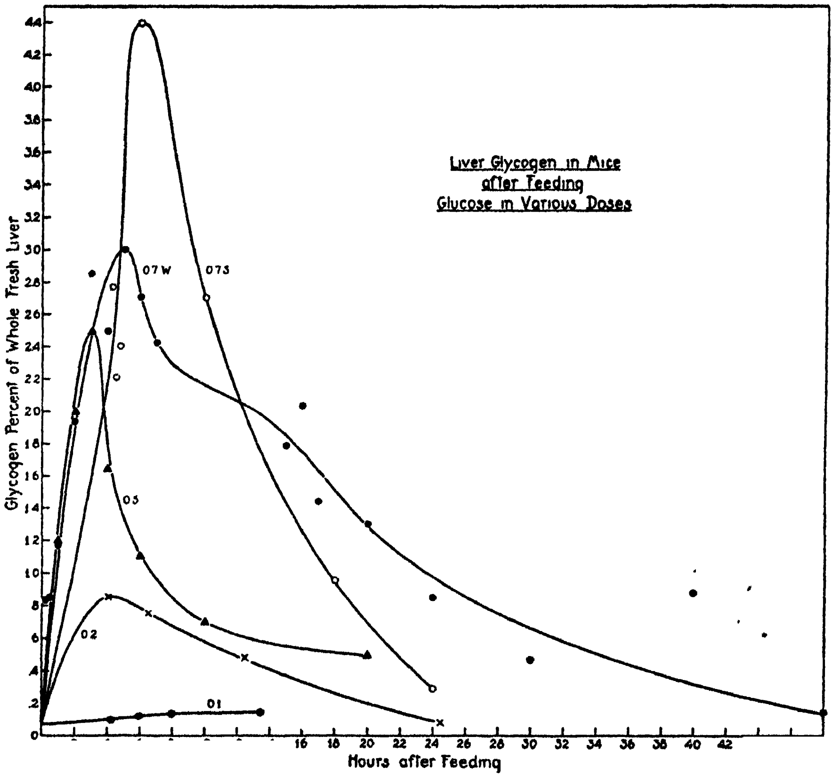

Fig. 1 The figures attached to each curve indicate the grams of glucose fed per 100 gm. of mouse. For 0.7 gm., both a winter and a summer curve are given.

EXPERIMENTAL DATA FOR SPECIAL CARBOHYDRATES

The results of the liver glycogen determinations after feeding derivatives of glucose are presented in table 3. Results are calculated in terms of total glycogen recovered, as well as in terms of the per cent of glycogen in the liver. The figures are plotted in figure 3 for comparison with the (similarly fed) molecular equivalents of glucose, given in figures 1 and 2.

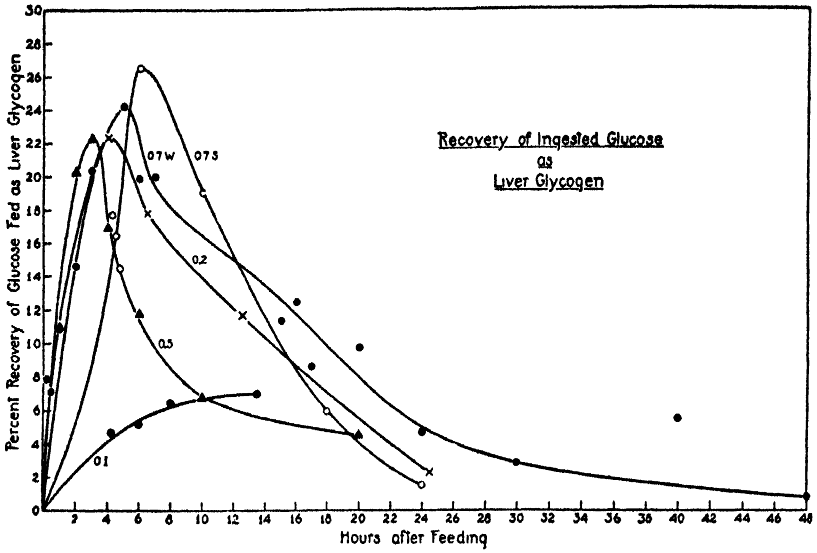

Fig. 2 The symbols attached to each curve have the same significance as in figure 1.

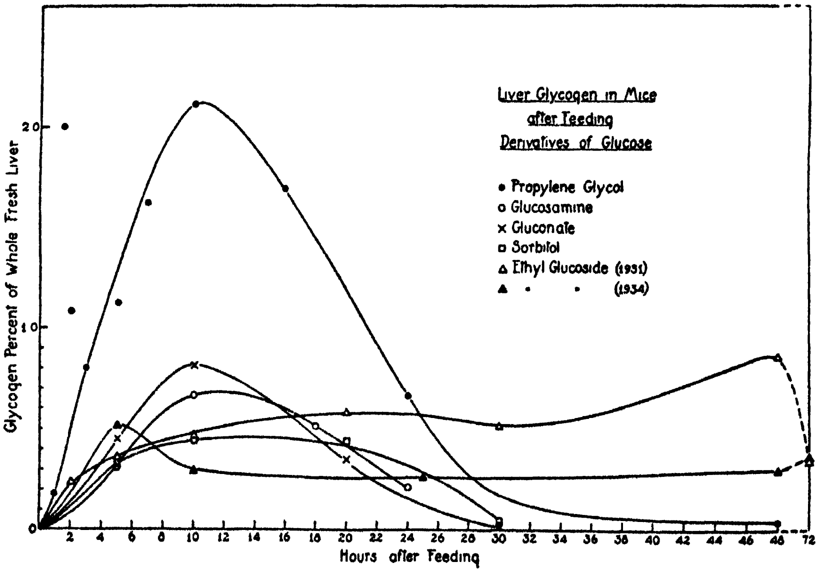

Fig. 3 The amounts of each carbohydrate fed are given in table 3. For ethyl glucoside, data are plotted as obtained in two separate years (1931 and 1934).

TABLE 3

Recovery of liver glycogen in mice after feeding derivatives of glucose

		TIME AFTER FEEDING	NUMBER OF MICE			AVERAGE GLYCOGEN CONTENT OF LIVER	AVERAGE FRACTION OF INGESTED CARBOHYDRATES
			Used	Dis-carded	Died		
		Hours				*Per cent*	*Per cent*
Propylene glycol feeding	Molecular glucose—equivalent: 0.75 gm. per 100 gm. of mouse	1	3			0.18	1.2
		1½	3			2.00	13.1
		2	9			1.08	7.8
		3	10			1.80	5.4
		5	8			1.12	9.6
		7	7			1.62	10.9
		10	4			2.11	15.4
		16	2			1.69	11.5
		24	8	1	1	0.66	4.4
		30	2			0.02	0.2
		48	2		6	0.04	0.1
Glucosamine feeding	Molecular glucose—equivalent: 0.370 gm. per 100 gm. of mouse	2	4				4.4
		5	6			0.32	4.6
		10	4			0.66	9.1
		18	3			0.51	7.0
		24	6	1		0.21	2.7
Gluconate feeding	Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse	5	4			0.45	23.9
		10	4			0.81	47.5
		20	6			0.35	18.7
		30	2		3	0.01	0.1
Sorbitol feeding	Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse	5	4			0.33	22.0
		10	2			0.44	25.2
		20	8			0.44	22.2
		30	2			0.05	2.2
Ethyl glucoside feeding (1931)	Molecular glucose—equivalent: 0.23 gm. per 100 gm. of mouse	2	5			0.24	3.1
		5	7			0.36	9.0
		10	3			0.46	13.4
		20	5			0.58	13.1
		30	4			0.51	11.9
		48	4			0.86	16.7
		72	2			0.35	8.7
Ethyl glucoside feeding (1934)	Molecular glucose—equivalent: 0.217 gm. per 100 gm. of mouse	5	4			0.51	13.5
		10	3		3	0.29	7.6
		25	7			0.26	3.3
		48	8		13	0.30	5.3
		72	3		5	0.36	7.0

Figure 4 shows the percentage recovery (in the liver) of the carbohydrate fed. This figure, like figure 3, describes increases in liver glycogen; but relates it to the amount of the carbohydrate fed, rather than to the weight of fresh liver in which it was stored.

Data are also presented in table 2 to illustrate the rate of absorption as evidenced by analysis of intestinal contents.

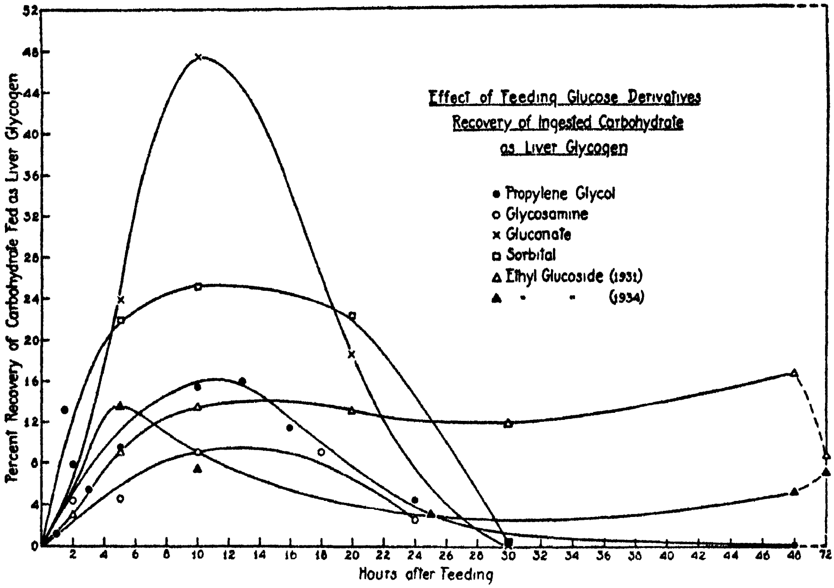

Fig. 4 These curves correspond to the data in figure 3, recalculated.

In the case of propylene glycol, the analytical method was the chromate oxidation described by Strebinger and Streit ('24) for the analysis of glycerine. The conversion factor was determined by analysis of known amounts of propylene glycol. This method is crude, because there was a large blank due to naturally occurring oxidizable matter in the intestine. The results are significant, therefore, only in indicating roughly how soon absorption was approximately finished.

DISCUSSION

It should be emphasized that these results apply only to the history of a single carbohydrate meal administered to fasting animals. The highest liver glycogen contents, here reported, do not attain the levels reported in the literature for continued high ingestion of carbohydrates. As might be expected, the concentration of glycogen in the liver rises higher when the glucose meal is larger.

One might expect a certain amount of glucose to be immediately burned. Indeed, our results with a very small glucose meal indicate very little storage in the liver. Above this level, however, it is not obvious why the fraction selected for deposition in the liver should remain a constant aliquot of the glucose fed.

Because of the limit imposed by gastro-intestinal tolerance, none of the substances here tested could be used in amounts as high as the maximal glucose ingestion. Nor is the storage of any of them better than that of glucose; indeed, two failed of as complete absorption as the glucose equivalent. Nevertheless, all showed undoubted evidence of glycogen storage, and its subsequent glycogenolysis as fasting proceeded. How much sugar was directly or indirectly utilized by the tissues at large was not determined in this work. It is believed, however, that the paying out of stored glycogen as fasting continued is good evidence that these substances are available as foods.

One word of caution is necessary as to the interpretation of the results. These substances were given to intact animals. The effect of intestinal action is, therefore, merged in the net effect produced. That sucrose, given parenterally, acts as a foreign substance is well recognized. It is conceivable that some of these compounds would not be utilized by an animal whose gastro-intestinal tract had been removed, even though the liver remained intact.

The effect of methyl glucoside is interesting. Its toxicity is apparent because the animals died an hour or two after feeding. Nevertheless, glycogen storage within the liver was indubitable a short time before death, as the analyses showed. The lethal effect is presumably due to progressive liberation of methyl alcohol. Similarly, the results obtained with ethyl glucoside were erratic, presumably because of the profound anaesthetic effect produced during the first day after feeding.

No attempt has been made in this paper to review the literature pertinent to these compounds, which have not been extensively studied as sources of energy for mammals. Drury and Salter ('34) have given references to a few such investigations, most of which have been concerned with the relief of insulin hypoglycaemia, or with organ perfusions.

SUMMARY

When the fasted mouse is fed a single large amount of appropriate carbohydrate, much of this is stored in the liver as glycogen. In the case of glucose, the maximal amount stored is roughly proportional to the amount fed, despite wide variation in the actual concentration of liver glycogen. As fasting proceeds, the stored glycogen is released from the liver. Standard curves are presented which illustrate this process in mice at various levels of glucose feeding.

For comparison, the storage and discharge of liver glycogen was followed after the ingestion of sugar derivatives in equivalent amounts. Five simple derivatives of glucose (sorbitol, gluconate, glucosamine, and the glucosides of methyl and ethyl alcohol) were studied, and one triose derivative (propylene glycol). All increased liver glycogen.

The results are compared with the effect produced by the molecular equivalent in glucose.

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THE EFFECT OF PHOSPHATES ON THE BONES OF RACHITIC RATS

C. A. LILLY, C. B. PEIRCE AND R. L. GRANT

Departments of Internal Medicine and Roentgenology, Medical School, University of Michigan, Ann Arbor, Michigan

THREE PLATES (TWELVE FIGURES)

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The earliest reference to the effect of phosphorus on bones was made by Wegner (1872). This investigator, working in Virchow's laboratory, stated that elementary phosphorus increased bone formation in growing animals and birds. Kassowitz (1884) claimed that elementary phosphorus stimulated repair of rachitic bones while Kissel (1896) reported no bone changes from the feeding of phosphorus. Phemister ('18) confirmed Wegner's (1872) and Kassowitz' (1884) observation that phosphorus was productive of bone formation. Wegner, Kassowitz and Phemister all fed elementary (red or yellow) phosphorus in daily doses of 1/100 to 1/400 of a grain. Sherman and Pappenheimer ('21) reported that the addition of alkaline potassium phosphate to a rachitiogenic diet (high in calcium and low in phosphorus) prevented rickets in rats when 0.4 per cent of the phosphate was added to, and an equivalent amount of calcium withdrawn from that diet. Hess and Unger ('22) and others have confirmed Sherman and Pappenheimer's observation. McCollum, Simmonds, and Shipley ('22 a) showed that the addition of 2 per cent of cod liver oil to a rachitiogenic diet, high in calcium and low in phosphorus, was curative of rickets in rats. Phemister, Miller and Bonar ('21) have reported that phosphorus, or phosphorus plus cod liver oil, produced similar (x-ray) results in bone development. Two of the children fed phosphorus only in addition to their previous deficient diet during the fall and winter showed roentgenologic signs of cure of marked rickets. McCollum, Simmonds, Shipley and Park ('22 b) reported that starvation of rats, made rachitic on a high calcium low phosphorus diet induced a cure of the rickets at the expense of other tissues of the body. Cavin ('24) reported that if rats were made to fast after having been made rachitic (on a high calcium low phosphorus diet) bone repair occurred, but convulsions and death followed. Hess and Weinstock ('26) disagreed with Wegner (1872), Kassowitz (1884) and Phemister ('18) with the statement that elementary phosphorus leads only to the production of 'phosphorus bands' at the epiphyses of the bones of rachitic rats and not to true healing. Karlitz and Shohl ('28) reported that the addition the salts of phosphorus (Na_2HPO_4) to a rachitiogenic diet (high in calcium and low in phosphorus), on which rats had been made rachitic, produced cure of the rickets, but in some was followed by carpopedal spasm, convulsions and death. Wilder ('28) questioned the findings of Karlitz and Shohl on the grounds that the animals studied by these authors "refused the phosphate diet to the extent of considerable loss of weight" and reported that the type of results shown can be produced by fasting as previously reported by McCollum et al. and Cavin. Lilly and Newburgh ('31) made a preliminary report that the addition of water-soluble extract of yeast and sodium phosphate (Na_2HPO_4) to a rachitiogenic diet (high in calcium and low in phosphorus), on which rats had been made rachitic, produced x-ray evidence of cure of the rachitic changes in such rats. Bloom ('32) reported that "the substitution of di-calcium phosphate for calcium carbonate in a rickets-producing diet resulted in the prevention and cure of rickets in albino rats." These conflicting opinions prompted us to further studies regarding the action of phosphates on rachitic rats.

EXPERIMENTAL STUDIES

Forty-five albino rats, 28 to 31 days old, were placed in a warm, well-ventilated, dark room. These animals were from the twelfth and thirteenth generations of our own in-bred rat colony. Their weights ranged from 65 to 85 gm. They were fed Steenbock and Black ('25) rachitiogenic diet no. 2965. Distilled water was given as desired. Thirty-three days later, x-ray examinations were made. All of the animals showed advanced rachitic changes. The animals were returned to the dark room and divided into three groups.

Group I. Fifteen rats (nos. 31–45) were continued on the Steenbock-Black rachitiogenic diet no. 2965.

Group II. Fifteen rats (nos. 1–15) were fed the Steenbock-Black rachitiogenic diet no. 2965 to which 0.03 per cent of irradiated ergosterol (viosterol 250 D) by weight was added and thoroughly rubbed up in the food. This gave this diet a vitamin D (curative) value equal to that shown by McCollum, Simmonds and Shipley ('22).[1]

Group III. Fifteen rats (nos. 16–30) were fed the Steenbock-Black rachitiogenic diet no. 2965 to which sodium phosphate (Na_2HPO_4) was added in amounts sufficient to give the diet a 0.04 per cent value in secondary phosphorus. This is a phosphorus value sufficient to prevent rickets in rats as shown by Sherman and Pappenheimer ('21).

These diets were continued for 33 days.

For the last 33 days of the test the average gain in weight for group I was 21.5 gm. per rat; for group II, 35.5 gm. per rat; and for group III, 46.5 gm. per rat.

One animal of group I died of an undetermined cause during the experiment. No convulsions, spasms or nervous irritability was observed in any group of animals.[2]

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[1] A standard viosterol (250 D) has a vitamin 'D' value 100 times that of a standard cod-liver oil.

[2] We have repeatedly observed carpopedal spasm and death in rats fed Steenbock's no. 2965, and on a few occasions convulsions, when the animals were losing weight, but have not observed these conditions when phosphates were added to the diet.

Roentgenologic observations. Roentgen examinations at the end of this second feeding period showed the following:

Group I. A greater x-ray evidence of rachitic changes than before.

Group II. X-ray evidence of healed rickets.

Group III. X-ray evidence of healed rickets equal to that of group II.

The entire series of rats on rachitiogenic diet no. 2965 at the end of the 33 days showed unmistakable roentgen evidence of active rickets with frayed bulbous metaphyses, lack of ossification of the growing bone with marked widening of the cartilaginous area between diaphysis and epiphysis, and some bowing of the long bones. The more marked changes, as would be expected, appeared at the wrists and knees of the animals. Similar changes were apparent at the proximal epiphyses of the humeri and femurs. The elbow was not so markedly involved, these epiphyses usually being well formed at an early period.

The group of controls which were continued on the rachitiogenic diet without corrective adjuvant showed progression of the deformities and non-ossification of the metaphyseal area. The cortical bone had not been notably disturbed. A specimen rat from this group is shown in the roentgenograms of plate 1.

The group of rats which received diet no. 2965 with irradiated ergosterol added demonstrated at the end of the 33-day period correction of the former rachitic interruption of bone growth. No marked cross striae of condensation were developed in the ends of the diaphyses. Some of the curvatures and widening of the transverse diameter of the metaphyseal portions of the shaft persisted, as would be expected. The epiphyseal growth disc again took on a smooth contour, of normal depth. The cortex of the shafts was relatively unchanged. A specimen rat from this series is shown in the roentgenograms of plate 2.

The third group which received diet no. 2965, but with the addition of sodium phosphate only, also showed at the end of the 33-day period on roentgen examination correction of the former rachitic disturbances. In these also the epiphyseal disc had resumed a normal depth, and smoother contour, the ossification of proximate portions of the long bones apparently normal. In these animals no notable increase in density of the diaphyses in cross-striae is found. The cortical bone remained normal in architectural pattern. Some of the previous deformities in the form of curvature and increase of the diameter of the metaphyseal portions of the shafts were of course, not corrected. A specimen rat from this group is shown in the roentgenograms of plate 3.

Chemical findings. At the conclusion of the experiment the rats were killed and the right femur removed from several animals in each group. The bones were cleaned mechanically to remove all loose tissue and were dried at 100° for 1 week. Each bone was broken, extracted in a Soxhlet apparatus, with alcohol for 10 hours and with ether for another 10 hours, and again dried. Each bone was ashed at a dull red heat in a muffle furnace and the percentage of total ash on the moisture-free weight determined. The ash was then dissolved in a dilute hydrochloric acid, made to a convenient volume, and aliquots were used for the estimation of calcium by the method of Tisdall and Kramer ('21) and of phosphorus by the method of Fiske and Subbarow ('25). The results are presented in the following table:

Ash of femurs

GROUP	ASH OF MOISTURE-FREE, FAT-FREE BONES	CALCIUM OF ASH	PHOSPHORUS OF ASH	$\frac{\text{CALCIUM}}{\text{PHOSPHORUS}}$
	per cent	*per cent*	*per cent*	
I	29.4	38.4	16.0	2.39
II	53.4	40.6	17.3	2.34
III	58.7	39.6	17.7	2.24

It is evident from these data that the femurs of the phosphate-treated rats (group III) are calcified to at least as great a degree as those of the rats receiving the viosterol (group II).

Histologic changes. At the conclusion of the feeding period one of the femurs of a rat from each group was prepared in the Department of Pathology for microscopic examination.

Comparative photomicrographs are shown in plates 1 to 3. The animal that remained on the unmodified diet, the original no. 2965, showed the typical rachitic fragmentation of cartilage and disorganization of the bone and cartilage. Both the animal receiving irradiated ergosterol and that receiving sodium phosphate added to the rachitiogenic diet demonstrated histologic evidence of previous rachitic change. There was some persistent irregularity in alignment of the cartilage columns of the epiphyseal disc and some residual fragmentation. In detail, however, the zone of primary calcification, the cartilage columns, and the resting cartilage had resumed normal proportions and a normal character of individual cells in both of the treated rats. The trabeculae of the shaft proximal to the zone of primary calcification in the phosphate specimen did not show appreciable difference from the irradiated ergosterol specimen.

The depth of osteoid tissue proximal to the epiphyses in the untreated animal was in striking contrast to the lack of a broad osteoid zone and the marked organization of new trabeculae in both treated specimens.

It is of note that the roentgen evidence of apparent cure is not completely supported by the histology of the tissue.

From the histologic standpoint the addition of basic sodium phosphate to the rachitiogenic diet corrects a rachitic animal equally as well as the administration of irradiated ergosterol.

DISCUSSION

Particular comment has been made above on the resumption of normal architectural pattern of the epiphyseal discs and the metaphyseal portions of the shafts without demonstrable sclerosis. Previous observers, notably Hess and Weinstock ('26), have called attention to a band of increased density of bone in a shaft which they attributed to phosphorus (the 'phosphorschicht'). This appeared, however, with the use of yellow phosphorus. Phemister ('18) also described a zone of increased longitudinal trabeculae in the ends of the shafts. It is of note that Phemister and his associates ('21) reported a complete correction of rickets in two severe cases by the addition to the previous dietary of 1/100 to 1/150 grain of phosphorus thrice daily. In contrast to the observations of Hess, our series of experimental animals which received sodium phosphate did not show 'phosphorus bands.'

Further, changes in the character of the cortical bone were described by earlier observers with the administration of phosphorus. We did not find such to be the case in the experimental series.

CONCLUSIONS

Rickets was produced in white rats by a diet high in calcium and low in phosphorus. The subsequent addition of phosphates to this diet, caused x-ray, chemical, and histological evidences of healing, at least equal to that caused by the addition of irradiated ergosterol (viosterol) to the diet of another group of rats previously made rachitic in the same manner. No neuropathic symptoms were noted in any animal of any group, and no 'phosphorus bands' were present in any specimen.

We wish to express our appreciation of the advice of Dr. L. H. Newburgh and the assitance of Dr. C. V. Weller during this experiment.

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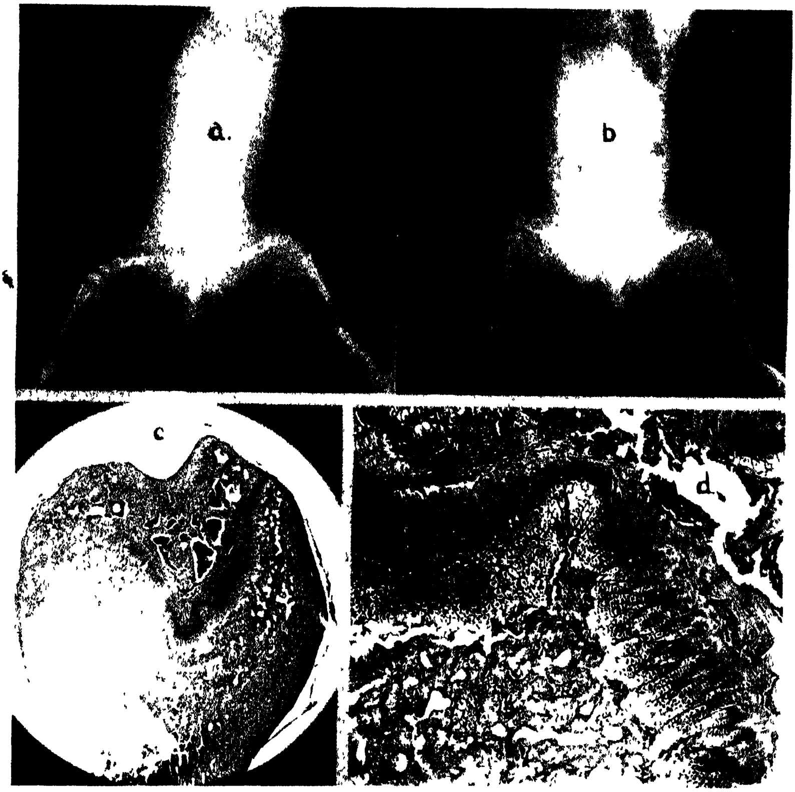


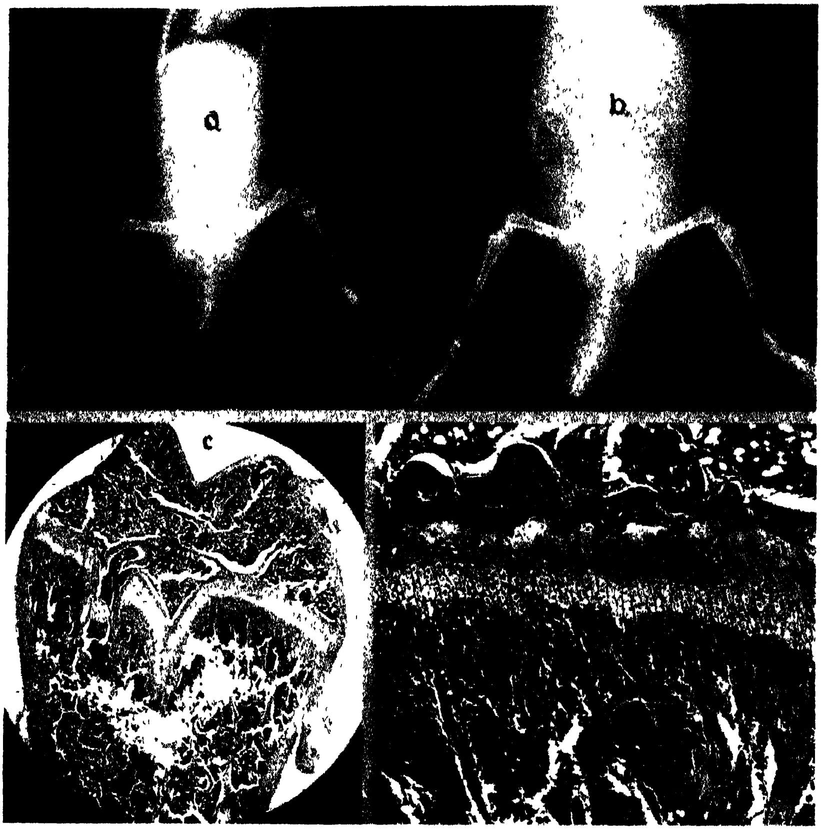
Rat no. 37 (group 1) control, on diet 2965 only.

a Roentgenogram after diet 2965 for 33 days (total age 64 days), active rickets.

b Roentgenogram after diet 2965 for 66 days (total age 97 days), progressive rachitic change.

c Photomicrograph (of longitudinal section of distal end of femur), showing deformity of epiphyseal cartilage zone, with increased depth, broad band of osteoid tissue proximal to cartilage.

d Photomicrograph (detail), showing depth and irregularity of cartilage, poor primary ossification, numerous osteoid trabeculae with poor marrow development.



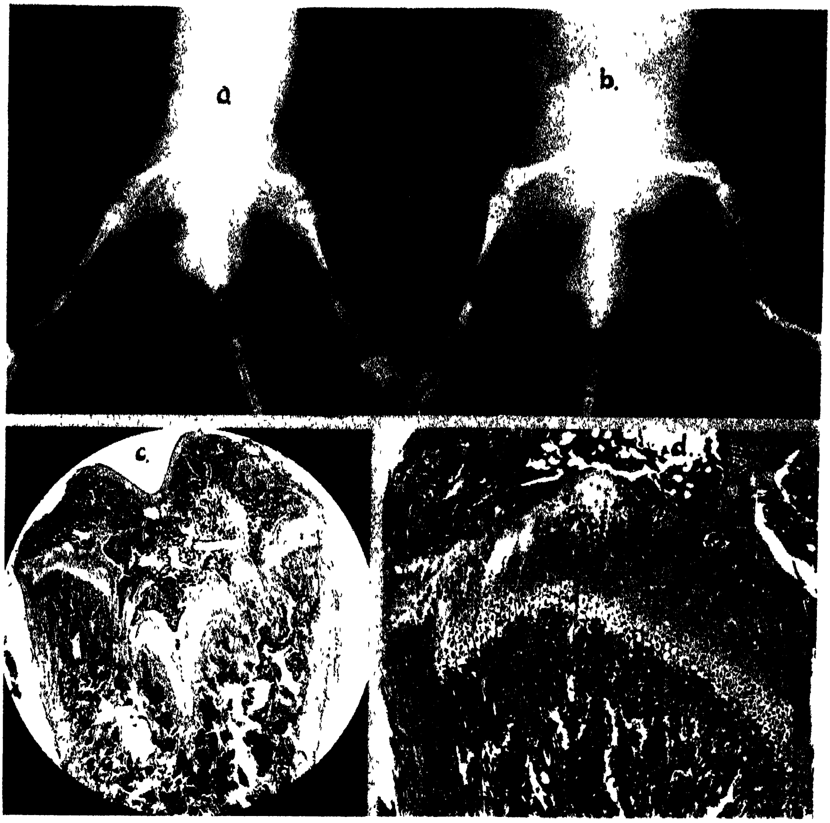
Rat no. 6 (Group II) diet 2965 only for 33 days, irradiated ergosterol (0.03 per cent by weight) added for 33 days.

a Roentgenogram at end of first period on rachitogenic diet. Comparable changes to that of the specimen of group I.

b Roentgenogram at end of second or corrective period. X ray evidence of cure of previous growth disturbance of epiphyseal junction.

c Photomicrograph of longitudinal section of femur (distal end). Residual irregularity of line of cartilaginous zone. But more normal depth than in group I. Normal depth of primary zone of calcification. No marked condensation of longitudinal trabeculae.

d Photomicrographic detail—good alignment and character of cartilage, normal zone of primary calcification, normal development of marrow spaces.



Rat no. 26 (group III) diet 2965 for 33 days plus sodium phosphate (Na_2HPO_4) for 33 more days.

a Roentgenogram at end of first period (diet 2965 only). Active rachitic changes at growth zones.

b Roentgenogram at end of second period (diet 2965 plus Na_2HPO_4) x-ray evidence of cure of rickets. Resumption of normal architecture at growing zones.

c Photomicrograph longitudinal section of distal end of femur residual irregularity of epiphyseal disc line—normal depth of zones—little variation from specimen of group II.

d Photomicrographic detail. No greater number of longitudinal trabeculae in metaphysis than in group II. No broad osteoid zone. Active growth of medullary area. Good resumption of normal cell alignment.

CATARACT IN RATS FED ON HIGH LACTOSE RATIONS

HELEN S. MITCHELL AND WARREN M. DODGE
Nutrition Laboratory, Battle Creek College, and Eye Department, Battle Creek Sanitarium, Battle Creek, Mich.

TWO FIGURES

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The occurrence of nutritional cataract in rats has been reported previously, but the etiological factors are not well established in spite of several contributions to the subject. At least three groups of workers have associated lens changes with a vitamin G deficiency. Day, Langston and O'Brien ('31), Langston and Day ('33) and Day and Langston ('34) conclude that cataract, conjunctivitis, and keratitis develop in gray or albino rats deprived of vitamin G in nearly 100 per cent of the cases. O'Brien ('32) and Yudkin ('33) have obtained cataract in animals deprived of vitamin G at an early date, whereas no cataract has developed in older animals. The latter concludes that at the present stage of our knowledge "we are not justified in attributing the formation of cataract to the lack of G alone." He reports furthermore that rats showed inflammatory and other ocular changes accompanying opacity of the crystalline lens. Curtis, Hauge and Kraybill ('32) have found lens opacities apparently due to a tryptophane deficiency and make no mention of vitamin G as a significant factor. A recent paper by Chaikoff and Lachman ('33) reports the occurrence of cataract associated with long-standing experimental diabetes in depancreatized dogs in which the ration had apparently been adequately supplied with protein and vitamins. The lens changes are attributed to the fact that the diabetic condition had existed over a considerable period of time.

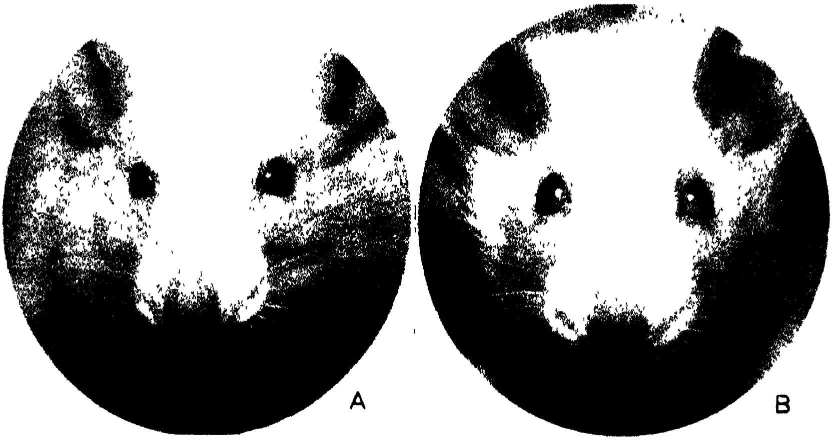
The various types of experimental cataract reported by these workers do not seem to point to any common etiological factors. The data reported in this paper suggest a metabolic disturbance rather than a vitamin or amino acid deficiency as the cause of the cataractous changes. Mature or markedly advanced cataracts have occurred in all rats fed a synthetic laboratory ration in which excessively high levels of lactose were the sole source of carbohydrate. Control animals fed on starch, maltose, dextrin or sucrose, respectively, as the only carbohydrate, in an otherwise similar ration, have never developed any ocular abnormalities. The accidental observation of mature cataract in the first three rats which had received a 70 per cent lactose ration in connection with another experiment stimulated the present investigation.

PROCEDURE

The rations consisted of 70 per cent carbohydrate, 15 per cent casein, 5 per cent (Osborne and Mendel) salt mixture, 9 per cent Crisco and 2 per cent cod liver oil fed ad libitum plus adequate daily doses of vitamin B complex in the form of 0.5 gm. dry brewer's yeast fed separately. Special precautions were taken to reinforce both the vitamin A and G supply to avoid these as complicating factors.

Rats on the 70 per cent lactose ration receiving four times the usual dose of dry brewer's yeast (2 gm. daily) developed cataract as soon as control rats from the same litter receiving the usual amount of yeast (0.5 gm. daily). Furthermore, there was no evidence at any time of a vitamin G deficiency. Growth was slightly subnormal on the 70 per cent lactose ration, but normal on the lower levels. All control rats on the other sources of carbohydrate grew normally with the 0.5 gm. of yeast as a daily supplement. The rats on the lactose rations appeared as sleek and well nourished as the control rats aside from the fact that they were blind. Young animals when first put on the ration often had a diarrhea which later subsided, although the stools frequently continued to be softer than those of rats on other carbohydrates. This condition might partially account for the retarded growth in young rats fed the 70 per cent lactose ration. The very lack of other striking abnormalities is in itself a significant finding.

Lest the lactose from one source might contain some unknown contaminant, a C.P. grade from another source was used in a limited number of cases with exactly the same results as those obtained with the less expensive grade of the sugar.



A, Normal rat. B, rat with cataract resulting from a 70 per cent lactose ration.

Beta-lactose was also used for comparison with the regular commercial form with the similar outcome. Thus it would appear that lactose, per se, must have been the significant factor in all cases.

Weekly ophthalmoscopic examinations were made on the eyes of both experimental and control animals. During this routine procedure the observer purposely remained ignorant of the ration of the specific animals, in order to avoid any unconscious prejudice in reporting findings. A detailed description of the ophthalmoscopic appearance of these lens opacities is significant. Oftentimes the earliest lens changes were noticed from one to several weeks before distinct opacities were observed and recorded. In tabulating results those animals which were not examined early, or those which were not recorded when first observed, are considered separately.

The development of cataractous changes may show considerable variation in type. The progressive lens opacities may begin first as a zone of defraction, a fine line in the lens around the periphery of the nucleus. As the beam of light is shifted by moving the ophthalmoscope from one position to another, the zone of defraction appears in one place and disappears in another. By this means the nucleus of the lens can be outlined in its entirety. The second step in this process is an increase in the size, width and density of this zone of defraction. This progresses with greater or lesser rapidity until there is often a ring of opacity outlining the entire nucleus of the lens, followed by a complete opacity of the nucleus if the progress is continuous.

A second form of lens change often found is a cortical opacity developing in the zonular region. This is located under the anterior lens capsule. The cortical opacities appear most frequently in the region of the ciliary body. First, one or several discrete flake opacities appear which frequently progress and coalesce until the entire lens is opaque. In a few of the eyes observed this opaque area appeared as a fine lace work, especially in those animals which had received an inceased amount of sodium chloride in the diet.

A third form of cataract development is a combination of the first and second. A zone of defraction develops and sooner or later cortical opacities also appear, or vice versa. The process may then continue to complete opacity or remain immature. This combination is by far the most common form of opacity development. In fact, those confined to cortical or nuclear portions of the lens alone are infrequent. In determining the type of opacity the ophthalmoscopic appearance alone was the criterion, no use being made of the slit lamp or the microscope.

Histological preparations were made of a few typical specimens by the celloidin method and stained with hematoxylin and eosin. The microscopic picture of the cataractous lens is typical. The microscopic appearance of the cornea, sclera, ciliary body and retina is normal, the same as that of the normal control eyes. Thus there is no evidence of the type of pathology usually associated with a vitamin A deficiency.

RESULTS

The summary of findings given in table 1 shows the number of mature and immature cataracts occurring in each ration

TABLE 1

Incidence of cataract in rats on different types and amounts of carbohydrates

RATION GROUPS	NUMBER OF RATS	AGE WHEN STARTED	PROGRESS OF CATARACT DEVELOPMENT							PERCENTAGE SHOWING	
			First appearance		Mature cataract		Immature cataract		None	Any lens changes	Mature cataract
			Number	Weeks	Number	Weeks	Number	Weeks			
		days									
70 % lactose	49	26	18 31	5.0[1] 1.3	33	10.0	16	22	0	100	68
70 % lactose	4	60	4	8.0	2	15.5	2	31	0	100	50
70 % lactose + Ca gluconate	5	26	5	2.4	3	13.0	2	12	0	100	60
50 % lactose	26	25	13 13	8.0[1] 2.4	7	17.0	18	34	1	96	27
50 % lactose	2	60	1	13.0			1	31	1	50	0
30 % lactose	6	23	6	3.6			6	37	0	100	0
30 % lactose	2	60							2	0	0
70 % starch	16	27							16	0	0
70 % dextrin	14	25							14	0	0
70 % maltose	4	23							4	0	0
50 % maltose	2	26							2	0	0
50 % sucrose	2	26							2	0	0

[1] Not examined during early weeks on the experiment.

group and the average number of weeks required for the development of these changes. Rats on the 70 per cent lactose ration showed progressive cataractous changes in all animals leading to mature bilateral cataract in 68 per cent of the cases. Rations containing lesser quantities of lactose resulted in slower development or a less pronounced type of lens change. Thus only 27 per cent of the rats on 50 per cent lactose developed mature cataract and none on the 30 per cent lactose ration. Of those developing mature cataract the average time required was 10 weeks on the 70 per cent ration and 17 weeks on the 50 per cent ration.

Practically all rats on the various lactose rations showed some lens changes. If those animals are disregarded which were not examined weekly from the beginning of the experiment there is a striking comparison between the level of lactose fed and the time required for the first appearance of early lens changes, namely, 1.3, 2.4 and 3.6 weeks, respectively, on the 70 per cent, 50 per cent and 30 per cent lactose rations. The lower the level of lactose fed, the longer was the time required for the development of lens changes.

This high incidence of cataract on the 70 per cent lactose ration and the literature relevant to the effect of lactose on calcium metabolism suggest several lines of investigation which would throw further light on this problem. An intensive preliminary study was made on a small group of animals: Twelve rats on the 70 per cent lactose ration and six controls were divided into five groups, as shown in table 2 and as many data as possible were collected on these groups. For calcium determinations on blood serum and eyes it was necessary to combine groups I and II and groups III and IV in order to have enough material for analysis. Two 24-hour urine samples from each rat were collected and tested for reducing sugar during the week before the animals were sacrificed. The Folin-Wu micro method was employed for urinary sugar and modified slightly for blood sugar determinations. Non-fasting blood sugars were high and irregular. The rats in the first five groups had had free access to food immediately before blood was taken for sugar determinations. Subsequent work on groups VI, VII and VIII showed that 12 hour fasting blood sugars ran much lower and entirely within normal limits for both the lactose and control groups. The urinary sugar was high in all of the groups fed on lactose; but group V, which had developed immature cataract showed less sugar than those with mature cataract. This observation on so small a group may not be significant. Further studies are in progress on the nature of the sugar excreted. Micro-chemical methods and osazone tests alone have proved unsatisfactory as means of differentiation.

TABLE 2

Metabolic changes associated with cataract

GROUPS AND CARBOHYDRATE CONTENT OF RATIONS		NUMBER OF RATS	FINAL AGE	FINAL BODY WEIGHT	INCIDENCE OF CATARACT	REDUCING SUGAR		ASH IN FAT-FREE FEMURS	CALCIUM CONTENT OF EYES MILLIGRAMS PER 100 GM.		SERUM CALCIUM MILLIGRAMS PER 100 CC.	
						Urine milligrams per 24 hours	Blood[1] milligram per 100 cc.		Moist	Dry	Total	Diffusible
			weeks	*gm.*				*per cent*				
I	70 % starch	3	24	192	None	2.7	122	65.4	8.4	35.0	10.5	4.5
II	70 % maltose	3	39	283	None	5.7	95(2)	65.2				
III	70 % lactose	4	30	148	Mature 17 weeks	34.2	177(3)	66.5	16.1	78.0	10.6	3.7
IV	70 % lactose	4	17	113	Mature 8.5 weeks	39.5	135(3)	61.5				
V	70 % lactose	4	32	212	Immature at end of 29 weeks	18.9	160	67.0	11.0	48.0	12.2	4.0
VI	70 % starch	8	30	232	None		88.0					
VII	70 % lactose	15	31	190	Mature and immature		74.8					
VIII	50 % lactose	14	35	257	Mature and immature		76.2					

[1] Groups I–V blood sugar determinations made without a preliminary fast

Groups VI–VIII blood sugar determinations made after a 12-hour fast

Qualitative or quantitative reducing sugar determinations before and after controlled fermentation periods have indicated the presence in the urine of a slowly fermentable sugar, such as galactose. The quantity of urine in young rats has made it impossible to run quantitative tests in all cases. These results plus the experience of other investigators yield exidence of galactosuria as a frequent result of high lactose ingestion.

Some of the data on calcium metabolism also seem to be significant. Total serum calcium and diffusible calcium do not show much deviation from the normal, although more determinations and on larger groups of animals should be made before definite conclusions are drawn. The calcium content of the cataractous eyes is about twice the normal, while that of eyes with immature cataract show less of an increase. That this was not merely a dehydration effect is shown by figures given on both moist and dry basis. Total ash of dry fat-free femurs was determined to see whether any significant bone changes were associated with this pathological condition. The constant high per cent ash in the bones of all groups indicates no significant effect there. This finding is not contradictory to that of Kline et al. ('32) on rachitic chicks, because our rats were not rachitic and calcification beyond the physiological optimum was not to be expected.

DISCUSSION

Inasmuch as high blood sugar in a diabetic is known to cause certain temporary lens changes and to predispose the older diabetic to cataract (O'Brien, '32 a), there appears to be a relationship between an excess sugar in the blood and the cataratous changes in the eyes. There is no evidence available regarding the influence of a specific sugar on the lens structure, but it is quite possible that traces of a foreign sugar in the blood, such as lactose or galactose, may have as detrimental an influence as an excess of glucose. McCance and Madders ('30) and Cori ('25) have shown that rats absorb galactose more rapidly than other sugars, while Folin and

Berglund ('22) have previously demonstrated a rapid excretion due to the lack of a renal threshold for galactose or lactose. Numerous other investigators have shown that these two sugars are unique in many of their metabolic processes. Further investigation as to the nature and extent of blood sugar changes in these animals will be significant.

It has been demonstrated by Bergeim ('26) and Kline et al. ('32) that lactose causes an increased absorption of calcium and possibly phosphorus from the intestinal tract. This specific influence of lactose on calcium absorption or upon its utilization has been repeatedly demonstrated in connection with the relief of parathyroid tetany (Dragstedt and Peacock, '23; Inouye, '24; Greenwald and Gross, '29). Tetany was relieved by lactose plus calcium salts, whereas calcium alone failed. It has also a decided favorable effect upon the calcification of rachitic bones in chicks (Kline et al., '32). Through its influence on the intestinal flora, this sugar is known to promote a relatively high pH through the entire length of the intestinal tract. The increased permeability of the intestinal membrane for certain minerals seems to be greater, however, than can be accounted for on the basis of the increased acid reaction of the intestine (Robinson and Duncan, '31; Sherman and Hawley, '22).

The logical result of the increased absorption would be a serum calcium higher than normal. If, however, a mere increase in blood calcium were the result of the lactose acting in the intestine one should be able to simulate this condition by intravenous injection of calcium salts. Such experiments have been tried on parathyroidectomized dogs without success (Adams, '29). Robinson et al. ('29) showed calcium retention from bone meal to be doubled by the use of lactose and concludes: "It seems plausible that lactose. . . . exerts a specific action in facilitating the passage of calcium into the blood stream, acting in this respect independently of the pH of the intestines." The fact that serum calcium was raised by calcium salts, but that calcium retention and the relief of tetany resulted only when lactose was also used indicated that there must have been some difference in the availability of the calcium circulating in the blood stream. The recent paper of Benjamin and Hess ('33) lends weight to such an hypothesis.

The inorganic constituents of cataractous human lenses have been investigated by Grabar and Nordman ('33), MacKay et al., ('32), Updegraff, ('32), Salit ('33) and Kirby ('30). It is significant that in all of these analyses the cataractous lenses contained more calcium than the normal, while the potassium was distinctly lower than normal in most cases; in fact, there was some alteration in proportion of several of the inorganic elements. In rat experiments the small size makes it more convenient to use whole eyes rather than the lenses for analyses. For this reason the calcium figures given in table 2 are less striking than they would be if less extraneous tissue had been included.

Changes in osmotic pressure which might result from an increased amount of sugar in the blood could scarcely account for this extensive migration of inorganic ions. If, however, the sugar increases the permeability of the delicate capsular membrane, such a shift in ions might be possible. This theory is supported by the observation that the mineral composition of the cataractous lens is much more nearly similar to the mineral composition of blood plasma than is that of a normal lens. Thus, if the normal protective action of the capsular membrane is destroyed, free exchange between plasma, aqueous humor and the lens substance might take place. It is well known that a puncture of the capsular membrane which admits aqueous humor will produce cataract. Thus the protoplasmic substance of the lens is apparently held in perfect solution by a nice balance of the inorganic ions present. If this balance is disturbed the proteins might be precipitated with the production of observed opacities.

The question as to whether animals other than rats are susceptible to cataract as a result of lactose feeding was investigated with kittens and rabbits. No lens changes were seen in any of these animals. The kittens developed such a severe diarrhea that the amount of lactose had to be reduced. The rabbits showed no lens abnormalities in 16 weeks on their skim milk-lactose ration in which the sugar constitutes 70 per cent of the dry weight. That rats may be peculiarly susceptible to cataract is suggested by the fact that lens changes occur in rats with a vitamin G deficiency, while cataract is not a characteristic symptom of a similar deficiency associated with pellagra in man.

The quantities of lactose used would never be approached in any but a laboratory diet. This fact coupled with the apparent susceptibility of the rat to cataractous development makes it impossible to apply these findings directly to the human or other animal species. On the other hand, the experimental production of cataract and its control by dietary means in a lower animal may become a valuable device for the more detailed study of cataractous development and accompanying metabolic changes.

SUMMARY

1. Mature bilateral cataract has occurred in 68 per cent of rats fed on a 70 per cent lactose ration with advanced lens changes showing in 100 per cent of the animals.
2. Rations containing lower quantities of lactose resulted in slower development or less pronounced type of lens change.
3. Cataracts have never appeared in rats fed starch, maltose, dextrin or sucrose, respectively, as the sole source of carbohydrate at the 70 per cent level.
4. The progressive lens changes have been described in detail. The developing opacities give the histological picture of human cataract.
5. Blood, urine, and tissue analyses on a limited number of animals indicated some disturbance in both carbohydrate and mineral metabolism with a loss of galactose in urine.
6. The calcium content of cataractous eyes doubles the normal. The total and diffusible calcium content of blood serum did not seem to be significantly altered.

7. The possibility of producing or controlling the development of cataract in lower animals by dietary means promises to be a valuable device for further and more detailed study of the etiology of cataract.

We are much indebted to Dr. Helen Sternberger, of the Children's Fund of Michigan, for calcium determinations on blood and eyes.

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THE EFFECT OF FERTILIZERS AND SOIL TYPES ON THE MINERAL COMPOSITION OF VEGETABLES[1]

J. M. COLEMAN[2] AND R. W. RUPRECHT

Department of Chemistry and Soils, Agricultural Experiment Stations, University of Florida, Gainesville

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Considerable interest recently has been manifested in the mineral content of food crops. This is especially true of such elements as calcium, iron, copper and manganese. In Florida there has long been a question as to whether the rather infertile soils produced crops low in mineral content or whether the heavy fertilization with commercial fertilizers produced crops having a high content of minerals. In order to throw some light on this subject, analyses of truck crops grown under known conditions of fertilizer treatment and on known soil types in various sections of Florida were undertaken.

The analyses reported in this paper were made largely on vegetables obtained from fertilizer experiments conducted in various sections of the State. As soon as possible after receipt of the samples they were washed and the edible portion was cut into small pieces and dried in an oven at about 75° C. When dry they were ground in a Quaker mill to a fine powder and stored in glass containers, tightly stoppered. All analyses are reported on a moisture-free basis.

The chemical methods employed were largely those of the American Association of Official Agricultural Chemists with some minor modifications. Copper was determined by the Biazzos method as modified by Elvehjem.

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[1] Presented before the Division of Agriculture and Food Chemistry at the 87th meeting of the American Chemical Society, St. Petersburg, Florida, March 26 to 30, 1934.

[2] Died February 18, 1934.

The figures for iron content are probably high, due to the fact that all of the materials were ground in an iron mill. At the start of these experiments the chief interest was in the other minerals rather than iron, hence no special precautions were taken in preparing the samples to avoid contamination with iron.

Table 1 (A) gives the analyses of tomatoes from plots differently fertilized. The first analysis shows the composition of tomatoes grown with a normal fertilizer application; the second, with a fertilizer containing only one-half the amount of phosphoric acid; the third, with a fertilizer containing a double amount of potash. Comparing the first two analyses, the only difference is in the phosphoric acid content, there being a slightly lower percentage where less was used in the fertilizer. Comparing the first and third analyses, no differences are found, especially none in the potash content, even though twice as much potash fertilizer was applied to those vegetables used in the third analysis. The last analysis shows the composition of tomatoes when the usual superphosphate in the fertilizer was omitted and colloidal phosphate, a very finely divided raw phosphate, was used as the source of phosphoric acid. In comparing this analysis with the first one, a lower content of phosphoric acid and of potash is found. As the phosphoric acid in the colloidal phosphate is less readily available than that in superphosphate, this may account for the lower phosphate content in the vegetables analyzed.

Table 1 (B) shows the analyses of lettuce variously fertilized. While there are some variations in the analyses, none can be attributed to the differences in the fertilizers used. As was the case with the tomatoes, changing the potash ratio in the fertilizer had no influence on the potash absorbed by the lettuce. The colloidal phosphate, even when used in double quantity, did not influence the mineral composition.

TABLE 1

Effect of fertilizers on composition of crops

FERTILIZER TREATMENT	ASH	CL	SO$_3$	P$_2$O$_5$	SIO$_2$	N	NA$_2$O	K$_2$O	CAO	MGO	FE PPM	MN PPM	CU PPM
A. Tomatoes													
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*			
Normal fertilizer	10.65	0.43	0.78	1.81	0.16	2.80	0.28	6.25	0.32	0.53	123	50	15
½ P$_2$O$_5$	10.25	0.46	0.81	1.59	0.18	2.69	0.16	6.18	0.29	0.50	193	44	11
2 × K$_2$O	10.45	0.51	0.84	1.74	0.12	2.70	0.32	6.13	0.30	0.50	172	47	10
Colloidal P$_2$O$_5$	10.39	0.50	0.67	1.60	0.13	2.84	0.30	5.99	0.30	0.49	214	52	12
B. Lettuce													
Normal fertilizer	17.09	1.31	1.23	1.74	1.00	3.59	0.34	7.75	1.75	0.20	480	177	29
½ K$_2$O	17.01	1.82	1.28	1.67	1.00	3.56	0.40	8.03	1.67	0.25	481	226	25
2 × K$_2$O	16.70	1.55	1.61	1.71	1.00	3.47	0.40	7.10	1.85	0.28	470	208	30
3 × K$_2$O	17.93	1.80	1.24	1.73	1.00	3.64	0.40	8.14	1.83	0.24	475	203	26
Colloidal P$_2$O$_5$	16.11	1.44	1.47	1.83	1.00	3.64	0.40	7.28	1.84	0.18	430	185	29
2 × colloidal P$_2$O$_5$	16.72	1.31	1.42	1.88	1.00	4.09	0.40	7.57	1.74	0.23	471	209	30
C. Celery													
Phosphoric acid	17.19	1.31	2.56	1.56	0.24	3.93	3.02	2.29	4.52	0.79	163	15	300
Potash	22.23	2.27	2.14	1.44	0.04	4.05	3.01	5.71	4.38	0.72	135	8	300
Phosphate and potash	21.68	1.70	2.64	1.49	0.05	3.61	2.37	4.87	4.67	0.76	150	18	293
Nitrogen, phosphate and potash	21.50	1.70	2.45	1.45	0.04	3.77	2.72	5.30	4.40	0.74	185	23	200
No fertilizer	18.66	1.73	2.27	1.25	0.14	3.91	2.90	2.51	4.41	0.77	130	13	413
Nitrogen, phosphate, potash and sulfur	19.97	1.45	2.78	1.38	0.03	3.69	2.44	5.00	3.91	0.69	130	30	140
Triple fertilizer application	19.22	1.24	2.26	1.82	0.08	4.08	1.59	6.27	3.41	0.67	240	10	150

Table 1 (C) shows the analyses of celery grown on Everglades muck soils with and without fertilizers. Comparing the celery grown on no-fertilizer plots with that grown on the phosphoric acid treated plots, there is a slight increase in sulfates and phosphoric acid in the fertilized celery. These increases are hardly significant, however, as there is almost as high a phosphoric acid content in the crop on the plot receiving only potash, and a lower sulfate content where a triple fertilizer application was made. Comparing the unfertilized celery with the potash-treated, a marked increase in the potash content is found; in fact, more than double the amount. Comparing the celery receiving phosphate and potash, the potash content is still high, but not quite so high as where potash alone was used. The only difference between the celery produced on the complete fertilizer plots and that from the no-fertilizer plots is in the higher potash content wherever this material was used. Tripling the fertilizer application brought about a slightly greater increase in potash and phosphoric acid. The copper content varies considerably. This is undoubtedly due to the heavy applications of Bordeaux spray and the inability to remove all traces of copper sulfate in preparing the samples for analyses.

In connection with some collaborative work with the American Association of Official Agricultural Chemists on the availability of phosphates, some interesting results were obtained. Soy beans were grown in quartz sand to which a nutrient solution without phosphoric acid was added. The phosphate was added separately in the form of superphosphate, mono-calcium phosphate and various ammoniated phosphates, at a basic rate equivalent to 400 pounds per acre of 18 per cent superphosphate. The results are shown in table 2. It will be noted that there was very little difference in the yield of dry materials. The phosphate analyses, however, show interesting differences. Taking first the superphosphate, a marked increase in phosphate content is found in soy beans which had received increased applications of phosphate fertilizer. The same is true where mono-calcium phosphate was the source of phosphoric acid. In the case of the ammoniated phosphates, decreasing amounts of phosphoric acid are taken up as the percentage of ammonia in the phosphate increases. The availability of the phosphoric acid in ammoniated superphosphate, as determined chemically, decreases in the same order.

INFLUENCES OF SOIL TYPES

The following tables show the effect produced by different soil types on the composition of vegetables grown on them.

As many readers may not be familiar with the soil types mentioned in the following pages a brief description of their general characteristics is given together with a table showing their average nitrogen, phosphoric acid and potash content.

TABLE 2

Effect of various phosphatic fertilizers on phosphate content of soy beans

SOURCE OF P_2O_5	DRY WEIGHT IN GRAMS	PER CENT P_2O_5 H_2O FREE
Superphosphate, ½ standard	5.5	0.47
Superphosphate, standard	6.5	0.82
Superphosphate, 2 × standard	6.0	1.34
Ammoniated superphosphate, 2.48 per cent NH_3	6.5	0.62
Ammoniated superphosphate, 5.36 per cent NH_3	7.0	0.50
Ammoniated superphosphate, 6.83 per cent NH_3	7.0	0.37
Ammoniated superphosphate, 8.66 per cent NH_3	7.0	0.29
Mono-calcium phosphate, ¼ standard	8.25	0.35
Mono-calcium phosphate, ½ standard	7.75	0.51
Mono-calcium phosphate, standard	8.50	0.93
Mono-calcium phosphate, 2 × standard	7.25	1.88

Bladen soil is normally a poorly drained soil containing a fair amount of organic matter and of plant nutrients. It is considered the best Irish potato soil in the State and is also used extensively for truck crops. The Glade marl is, as its name implies, made up largely of finely divided marl (90 to 95 per cent calcium carbonate) overlain with a layer of mucky material from 3 to 12 inches deep. These lands are usually flooded during the summer and early fall, and thus undergo a thorough leaching each year. The Hernando soils are among the more fertile soils in the State, containing slightly more plant food and organic matter than do the Bladen.

The Coxville soils are very similar to the Bladen, but have somewhat better natural drainage and a more plastic subsoil. The Norfolk soils are the predominating soils of the high lands of the State. They are low in humus or organic matter and low in plant nutrients. The Fellowship soils are very similar to the Hernando soils. The Leon soils are naturally among the poorest in the State. They are underlain with a hardpan layer and are low in plant foods. However, when sub-irrigation and drainage systems are installed and heavy fertilizer applications made, they produce bountiful crops.

Saw-grass muck soils are the predominating type found in the Everglades. They are soils in which the organic matter has not been decomposed very much. Technically speaking, they are peats rather than mucks. They are not productive when first put into cultivation unless given special treatment with salts of copper, manganese and zinc. The Parkwood soils are poorly drained soils underlain with marl. They produce excellent crops when drained, as they are among the richest soils in plant food that we have in the State.

TABLE 3

Content of nitrogen, phosphorus and potassium in soil types

SOIL	NITROGEN (N)	PHOSPHORIC ACID (P_2O_5)	POTASSIUM OXIDE (K_2O)
Bladen	0.107	0.027	0.048
Hernando	0.198	0.080	0.047
Norfolk	0.050	0.019	0.011
Leon	0.087	0.007	0.009
Parkwood	0.272	0.462	0.054
St. Lucie	0.025	0.012	0.006

Table 4 (A) shows analyses of tomatoes grown on Bladen, Glade marl and Hernando soil types. The differences in composition of the tomatoes are not great. The tomatoes grown on Hernando soil have a higher ash content and higher chlorine and sodium content, undoubtedly due to a higher sodium chloride content in the soil which lies near the coast.

TABLE 4

Effect of soil type on composition of crops

SOIL TYPE	ASH	CL	SO$_3$	P$_2$O$_5$	SIO$_2$	N	NA$_2$O	K$_2$O	CAO	MGO	FE PPM	MN PPM	CU PPM
A. Tomatoes													
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*			
Bladen	11.00	0.48	0.80	1.73	0.13	2.81	0.29	6.17	0.31	0.51	159	51	11
Glade marl	11.72	0.79	0.88	1.49	0.02	3.71	0.25	7.06	0.48	0.61	171	70	15
Hernando	12.64	0.91	0.78	1.85	0.55	3.04	0.61	6.51	0.63	0.51	133	28	22
B. Potatoes													
Coxville	5.04	0.22	0.60	0.68	0.20	1.45	0.24	2.45	0.31	0.35	94	27	13
Bladen	5.27	0.47	0.59	0.76	0.13	1.16	0.27	2.66	0.32	0.31	76	21	22
Norfolk	5.40	0.39	0.58	0.61	0.15	1.46	0.19	3.14	0.33	0.38	69	11	10
Fellowship	4.46	0.41	0.74	0.69	0.09	2.45	0.23	2.24	0.29	0.36	65	10	10
Leon	5.41	0.43	0.71	0.90	0.37	2.15	0.20	2.88	0.26	0.29	71	11	20
C. String beans													
Muck	7.88	0.43	0.92	1.03	0.56	4.31	0.19	2.92	1.33	0.72	145	23	25
St. Lucie	7.74	0.42	0.85	1.53	0.15	3.30	0.27	2.89	1.39	0.65	146	51	13
Parkwood	7.00	0.50	0.93	1.11	0.29	3.38	0.25	1.80	0.90	0.09	168	22	14
D. Cabbage													
Leon	8.74	0.44	2.52	1.52	0.26	3.01	0.31	3.50	1.14	0.05	160	31	21
Saw-grass muck	9.00	0.92	2.60	1.55	0.29	2.55	0.25	4.00	1.60	0.04	120	28	11
Coxville	8.05	0.46	2.69	1.17	0.14	2.78	0.44	3.84	0.81	0.04	280	34	15
Parkwood	10.06	0.74	2.27	1.28	0.12	3.16	0.30	4.42	1.83	0.04	154	29	10

It is worthy of note that tomatoes grown on marl had a lower calcium content than those grown on Hernando soil. The lower amount of phosphoric acid in the tomatoes grown on marl is probably due to the high pH of this soil which depresses the availability of the phosphoric acid. The higher manganese content of the marl tomatoes is due to the use of manganese in the fertilizer.

Table 4 (B) shows the analyses of potatoes grown on various soil types. In comparing these analyses very little differences are found. The chief difference is in the nitrogen content of the potatoes grown on the Fellowship and Leon soils. Just why these should be so much higher is difficult to explain. The higher manganese content of the potatoes grown on the Coxville and Bladen soils is due to the inclusion of manganese in some of the fertilizers used on these plots.

Table 4 (C) gives the analyses of string beans growing on Everglades muck, St. Lucie sand and Parkwood sandy loam. The St. Lucie soils are about the poorest soils from an agricultural standpoint that we have in the State, being very low in natural fertility and in organic matter or humus. However, analyses of beans growing on this infertile soil show that when fertilized it produces crops having as high a mineral content as when grown on the rich Everglades muck soil, and higher than when grown on Parkwood soils which naturally are more fertile soils.

Table 4 (D) shows cabbage grown on a variety of soil types. Here again the differences in composition are not outstanding, with one or two exceptions. The cabbage grown on muck and Parkwood soils carries more chlorine. Both of these soils are located on the east coast, while the other two soils are located in the central section of the State. The phosphoric acid content is somewhat lower in cabbage grown on the Coxville and Parkwood soils; nitrogen is higher in cabbage produced on Leon and Parkwood, while calcium is very low in the Coxville cabbage.

The claim has been made that Florida grown vegetables contain a higher water content than those grown farther north and that, therefore, it takes more of them to supply the same amount of nutrients. Table 5 shows the moisture content of vegetables grown in Florida compared with those reported by Peterson and Elvehjem ('28). With the exception of strawberries and beets, the agreement between the two sets of figures is remarkably close.

As was stated in the beginning of the paper, there has been considerable doubt in the minds of many as to the mineral content of truck crops grown in Florida. In table 6 are shown comparisons of the mineral content of Florida grown vegetables with analyses reported by Sherman ('32). In comparing these analyses it must be borne in mind that the analyses reported by Sherman were made a good many years ago. Refinements in the methods of analyses probably account for some of the differences. This is undoubtedly true of the figures for sulfur. A remarkable feature of this table is the close agreement in almost every case of the figures for potash. In the majority of cases Florida grown crops have a higher mineral content than those shown in Sherman's tables. This is especially true of calcium and phosphoric acid, two elements that are receiving considerable attention from nutrition specialists.

TABLE 5

Moisture content of vegetables

	FLORIDA	PETERSON
	Per cent	*Per cent*
Beans	90.89	91.40
Carrots	89.33	90.10
Cabbage	92.73	92.60
Celery	92.54	94.00
Lettuce	94.83	94.40
Potatoes	79.76	78.20
Tomatoes	95.20	94.20
Beets	88.85	82.30
Peppers	93.68	94.00
Strawberries	94.01	90.30
Turnips	91.76	91.50

The last table, 7, compares the copper and manganese found in vegetables grown in Florida with that found by other workers. The Florida analyses were, with one exception, made of crops that had received no manganese or copper as fertilizer. The exception was celery, which had all been fertilized with copper sulfate. Of course, practically all of the

TABLE 6

Ash constituents in fresh materials in per cent of edible portion

	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3	Cl	
Beans (string)	0.064	0.041	0.298	0.026	0.119	0.075	0.024	Sherman
	0.115	0.053	0.281	0.024	0.124	0.081	0.040	Florida
Beets	0.041	0.035	0.425	0.125	0.089	0.040	0.058	Sherman
	0.074	0.060	0.440	0.159	0.123	0.087	——	Florida
Cabbage	0.063	0.025	0.298	0.036	0.066	0.165	0.024	Sherman
	0.083	0.004	0.266	0.022	0.116	0.183	0.032	Florida
Carrots	0.078	0.035	0.346	0.136	0.105	0.055	0.036	Sherman
	0.099	0.047	0.472	0.091	0.157	0.093	0.057	Florida
Celery	0.109	0.023	0.381	0.113	0.085	0.055	0.156	Sherman
	0.328	0.055	0.395	0.203	0.108	0.183	0.013	Florida
Lettuce	0.060	0.028	0.408	0.036	0.096	0.035	0.074	Sherman
	0.090	0.010	0.400	0.018	0.090	0.064	0.068	Florida
Peppers	0.008	0.017	0.167	——	0.060	0.035	0.013	Sherman
	0.031	0.027	0.280	——	0.078	0.061	0.025	Florida
Potatoes	0.020	0.046	0.517	0.028	0.133	0.075	0.038	Sherman
	0.061	0.068	0.540	0.047	0.148	0.129	0.077	Florida
Strawberries	0.057	0.032	0.177	0.067	0.064	0.035	0.006	Sherman
	0.049	0.051	0.202	0.022	0.062	0.043	0.019	Florida
Tomatoes	0.015	0.017	0.331	0.013	0.060	0.035	0.034	Sherman
	0.038	0.044	0.542	0.022	0.139	0.068	0.038	Florida
Turnips	0.090	0.028	0.407	0.075	0.105	0.162	0.041	Sherman
	0.137	0.019	0.353	0.096	0.114	0.167	0.091	Florida
Eggplant	0.015	0.025	0.169	0.013	0.078	0.040	0.024	Sherman
	0.031	0.035	0.265	0.035	0.105	0.055	0.057	Florida
Onions	0.048	0.027	0.214	0.022	0.103	0.175	0.021	Sherman
	0.097	0.035	0.269	0.041	0.130	0.181	0.038	Florida

vegetable crops had been sprayed several times with Bordeaux mixture, which may account in part for the higher copper

TABLE 7

Manganese and copper content of vegetables
(ppm. in moisture free material)

	Mn				Cu		
	Lindow and Peterson	Remington and Shiver	Richards	The authors	Lindow Elvehjem Peterson	Remington and Shiver	The authors
Beets	35.1–76.2	55.4	21.7	23.5	11.5	9.1	10–25
Cabbage	10.6	40.7	15.0	23–58 Av. 30	6.8	6.3	10–28 Av. 17.8
Carrots	6.0	42.2	16.7	25–40	8.1	10.7	10–20
Celery	27.1			8–67 Av. 32	2.0		100–1600
Beans (string)		35.2		20–25	12.0	9.6	7–20
Eggplant		31.1		40–50	13.6	12.7	7–16
Lettuce	216 N 100 S	118.4	133	141–345 Av. 197	11.6	10.9	18–33 Av. 28.6
Onions	7.9	63.3	9.8	50–62	13.4	11.5	13–16
Peppers	18.2			13–70	16.1		8–18
Potatoes (Irish)	4.5	9.4	14.3	5–21 Av. 13.7	8.0	7.4	4–28 Av. 16.4
Potatoes (sweet)	5.4 19.6	9.3		8	5.2	6.2	8
Tomatoes		25.7		20–65 Av. 48	9.9	17.4	6–24 Av. 11.7
Turnips		13.6	3.6	9.5	11.0	4.4	14

content. Both copper and manganese are fully as abundant in vegetables grown in Florida as in those grown in other sections of the country. We found, as others have found, that the content of copper and manganese varies greatly in samples of the same vegetable grown in different sections of the State. The content of both of these elements can be increased through the use of manganese sulfate or copper sulfate in the fertilizer.

It will be extremely interesting to study the composition of crops produced through the continued use of the new synthetic and highly concentrated fertilizers. Considering the small amount of some of the secondary plant nutrients contained in these fertilizers, and the small amounts of these elements present in Florida soils, the time may come when this combination will bring about a lowered mineral content of the crops and also a lowered yield. The results reported in this paper indicate the following:

SUMMARY

1. That complete fertilizers containing nitrogen, phosphoric acid and potash, when used in the usual amounts necessary for optimum crop production exert very little influence on the composition of the crops grown with them.
2. Heavy applications of fertilizer will nullify any effects which soil types may have on the composition of the crop.
3. Florida grown vegetables are as high in minerals, including copper and manganese, as vegetables produced in other sections of the country; in some cases their mineral content is higher than that of vegetables from elsewhere.

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THE STABILITY OF VITAMINS $B(B_1)$, $G(B_2)$ AND B_4[1]

J. A. KEENAN, O. L. KLINE, C. A. ELVEHJEM AND E. B. HART
Department of Agricultural Chemistry, University of Wisconsin, Madison

ONE FIGURE

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A study of the heat stability of vitamins $B(B_1)$ and $G(B_2)$ in natural grain diets using the chick as the experimental animal has been reported by Elvehjem, Kline, Keenan and Hart ('32). With the knowledge of the intense requirement of this species for vitamin B_4 as reported by Keenan, Kline, Elvehjem and Hart ('33) it was decided to extend our knowledge to the heat stability of this factor and to investigate further the stability of the antineuritic and antipellagric components of the vitamin B complex.

We have tested the stability of these factors in a natural grain diet, in a commercial yeast, and in a hog's liver preparation. It is our purpose in this paper to discuss, 1) the heat stability of vitamins B, G and B_4 in the foodstuffs mentioned; 2) the separation of these factors by differential heat treatment, and from this knowledge, 3) the formulation of basal diets suitable for assaying foodstuffs for their antineuritic, antipellagric, or antiparalytic properties.

EXPERIMENTAL

White Leghorn chicks were used exclusively as experimental animals. The basal grain diet, and the method used for housing and feeding these birds throughout the experimental period was the same as that previously described by Elvehjem,

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[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

Kline, Keenan and Hart ('32). The yeast (baker's) was obtained fresh from the Red Star Yeast Company in Milwaukee and dried at 65°C. before using. The liver was a vacuum desiccated product obtained from the Wilson Laboratories, Chicago, and designated as 'special whole liver substance (Wilson).'

The heat treatments of the grain ration, yeast, and whole liver substance were all made at their natural hydrogen ion concentration. The pH is therefore constant, the medium, degree, and time of heating being the variables. The presence of moisture in the medium such as obtains in the autoclave, by heating a substance in the fresh state or by moistening a previously dried foodstuff is designated as treatment in a moist medium. The dry medium entails the treatment of a dry or relatively dry foodstuff in an electrically heated oven.

THE STABILITY OF VITAMIN B(B_1)

When the natural grain diet is fed to chicks they attain an average growth of 320 to 350 gm. by the sixth week. Autoclaving this diet, however, inactivates the vitamin B completely and polyneuritis is demonstrable in 7 to 9 days. With the incorporation of 4 per cent of the dried Red Star yeast or 18 per cent whole liver substance into the autoclaved diet, a growth is obtained comparable with that of the untreated ration. The heated liver and yeast were incorporated into the autoclaved ration at these same levels and at higher levels to determine the degree of destruction.

Table 1 has been compounded from a series of determinations made upon these foodstuffs subjected to various heat treatments. Complete inactivation of the vitamin is indicated by the negative symbol, whereas one, two or three positive signs have been used to demonstrate the relative degree of destruction which is determined by the deviation from the normal growth curve.

In the previous paper by Elvehjem, Kline, Keenan and Hart ('32) the observation was made that the antineuritic factor was stable in the grain ration when heated dry at

100°C. for 1 day, but if this treatment was prolonged for 6 days some inactivation did occur. More vitamin G is inactivated in this process, however, for symptoms of pellagra are observed. Raising the temperature to 110°C. and maintaining this treatment for 6 days does not cause a greater destruction of vitamin B. At 120°C. This factor is stable for a period of 5 hours, is slightly destroyed if the treatment is prolonged for a day, and completely inactivated at this temperature for 6 days.

TABLE 1

The heat stability of vitamin $B(B_1)$

SUBSTANCE TESTED	pH	HEAT TREATMENT			VITAMIN B STABILITY
		Medium	Temperature	Hours	
Ration	5.5–6.0	Moist	Autoclave	5	—
Yellow corn 58	5.5–6.0	Dry	100°C.	144	++[1]
Wheat middlings 25	5.5–6.0	Dry	110°C.	144	++[1]
Casein 12	5.5–6.0	Dry	120°C.	5	+++
NaCl 1	5.5–6.0	Dry	120°C.	24	++[1]
	5.5–6.0	Dry	120°C.	144	—
Yeast	6.0–6.5	Dry	120°C.	24	++
Yeast	6.0–6.5	Moist	Autoclave	5	—
Yeast	6.0–6.5	Dry	120°C.	144	—
Liver	6.0–7.0	Moist	100°C.	24	—
Liver	6.0–7.0	Dry	100°C.	24	+++
Liver	6.0–7.0	Dry	120°C.	24	++

[1] Symptoms of pellagra.

The antineuritic potency of yeast and liver is lowered slightly by a dry heat treatment of 120°C. for 24 hours. The previous observation that heating fresh yeast at 100°C. for 1 day would inactivate the vitamin B may now be extended to liver. The antineuritic value of the liver and yeast may be retained, however, by drying at 65°C. prior to heating at 100°C. The vitamin B in the ration, yeast and liver is therefore completely inactivated by autoclaving and destroyed to a large extent in yeast and liver by heating fresh at 100°C. for 24 hours. However, in the absence of moisture, the temperature may be increased to 120°C., with very little destruction.

THE STABILITY OF VITAMIN G(B_2)

The natural grain diet which has been heated to 100°C. for 144 hours has been used as a basal diet in assaying the yeast and liver for their antipellagric properties. Chicks reared upon this diet will develop symptoms of pellagra in 3 weeks. The incorporation of 2 per cent yeast or 1 per cent whole liver substance with this ration will supply all requirements of the chick for this factor.

TABLE 2

The heat stability of vitamin G

SUBSTANCE TESTED		pH	HEAT TREATMENT			VITAMIN G STABILITY
			Medium	Temperature	Hours	
Ration		5.5–6.0	Dry	100°C.	144	—
Yellow corn	58	5.5–6.0	Dry	120°C.	24	—
Wheat middlings	25					
Casein	12					
NaCl	1					
Yeast		6.0–6.5	Dry	100°C.	144	+
Yeast		6.0–6.5	Dry	120°C.	24	—
Yeast		6.0–6.5	Moist	100°C.	24	+++
Yeast		6.0–6.5	Moist	Autoclave	5	+++
Liver		6.0–7.0	Dry	100°C.	144	+
Liver		6.0–7.0	Dry	120°C.	24	—
Liver		6.0–7.0	Moist	Autoclave	5	+++
Liver		6.0–7.0	Moist	Autoclave	10	++

From table 2 it will be observed that there is as great a destruction of the vitamin G in the ration by heating at 120°C. for 24 hours as is accomplished by the 144-hour heating at 100°C. The latter heating process inactivates approximately 50 per cent of the vitamin G contained in yeast and liver. However, a very large part of the antipellagric factor may be inactivated in the yeast and liver by the heat treatment of 120°C. for 1 day—a process which does not alter the vitamin B content appreciably. For this procedure, the grain ration is placed in small pie pans in layers about ½ inch thick. When the electric oven is filled with the ration, approximately 5 hours is required for the temperature to attain 120°C., so that the ration is heated only 19 hours at this temperature.

THE STABILITY OF VITAMIN B_4

The vitamin B_4 activity of the ration and liver was tested by incorporating them into a synthetic diet which we have shown to be deficient in this factor (Keenan, Kline, Elvehjem and Hart, '33). It has been found recently that autoclaving the casein of this diet will remove the last remaining traces of the antiparalytic factor which makes the ration a more drastic one for B_4 assay studies. This synthetic ration which carries the autoclaved casein is designated diet no. 441A. The yeast, because of its very low antiparalytic activity, was not assayed. Table 3 designates the various heat treatments to which the grain ration and liver were subjected.

The inclusion of 32 per cent of the natural grain diet (ration 240) into the synthetic ration renders this diet complete for the chick. The grain diet which has been heated to 100°C. for 6 days has been used as a basal G low diet. Examination of table 3 indicates that all but a small portion of the vitamin B_4 is destroyed by this treatment. This emphasizes the inadequacy of this diet in testing a foodstuff for its antipellagric properties because of its deficiency in the antiparalytic factor. However, treatment of the basal diet at 120°C. for 24 hours does not alter the vitamin B_4 content appreciably, although it is extremely low in vitamin G, hence its applicability for vitamin G assay studies.

It will be observed (table 3) that there is some inactivation of the vitamin B_4 in dried whole liver by moistening and again drying at 65°C. for 24 hours. Autoclaving the liver completely destroys the vitamin B_4 contained therein, but by subjecting the natural grain diet to this treatment the greater portion of this factor is retained. It is therefore apparent that the presence of moisture catalyzes the destruction of vitamin B_4 as well as that of vitamin B, as shown by Elvehjem, Kline, Keenan and Hart ('32) yet in the autoclave both the ration and liver are heated in an equivalent medium. This suggests the greater lability of vitamin B_4 in liver as compared to grains when treated under similar conditions.

TABLE 3

The heat stability of vitamin B_4

RATION NO.	NUMBER CHICKS	SUPPLEMENT	HEAT TREATMENT OF SUPPLEMENT	AVERAGE WEIGHT 6 WEEKS	NUMBER WITH PARALYSIS	DEATHS AT 6 WEEKS	VITAMIN B_4 ACTIVITY
441 A	7	None	None		7	7	—
441 A	7	32 per cent ration 240	None	365	0	0	+++
441 A	7	32 per cent ration 240	100°C.—144 hours (G low diet)	...	6	5	+
441 A	7	32 per cent ration 240	Autoclave—5 hours (B low diet)	255	3	0	++
441 A	7	32 per cent ration 240	120°C.—24 hours dry (G low diet)	322			++
441 A	7	18 per cent whole liver		350			+++
441 A	2	18 per cent whole liver	Moistened and dried at 65°C.—24 hours	315			++
441 A	7	18 per cent whole liver	Moistened and dried at 100°C.—24 hours		7	7	—
441 A	7	18 per cent whole liver	Autoclaved—5 hours		7	7	—

THE DIFFERENTIAL DESTRUCTION OF VITAMINS B, G AND B_4

A comparison of the heat stability of these factors is given in table 4, which embodies the observations which have been made on the grain ration, the yeast, and the liver. Using a moist medium, all the factors are relatively stable at 65°C. for 24 hours. When the temperature is increased to 100°C. for the same length of time the antineuritic and antiparalytic components are inactivated. Autoclaving the ration inactivates vitamin B, but a greater portion of vitamin G and B_4 are retained. Autoclaving liver or yeast, however, destroys the antineuritic and antiparalytic factors, but saves vitamin G.

Using a dry heat all factors are stable at a temperature of 100°C. maintained for 24 hours. Prolonging this treatment for 6 days inactivates vitamins G and B_4 but the antineuritic factor is relatively stable. A treatment of 120°C. for 24 hours will inactivate G completely and retain the greater portion of vitamins B and B_4.

Thus vitamin B may be destroyed and the greater portion of vitamin G and B_4 retained by autoclaving the grain ration for 5 hours at 15 pounds' pressure. Vitamin G is inactivated and the other fractions preserved in the grain ration by a dry heat treatment of 120°C. for 24 hours. A portion of the antiparalytic potency of liver is lost by moistening and drying at 65°C. for 24 hours.

TABLE 4

Heat stability of vitamins B, G and B_4

HEAT TREATMENT			VITAMIN G	VITAMIN B	VITAMIN B_4
Medium	Temperature	Hours			
Moist	65°C.	24	+++	+++	++
Moist	100°C.	24	—	+++	—
Moist	Autoclave (ration)	5	—	+++	++
Moist	Autoclave (liver)	5	—	+++	—
Dry	100°C.	24	+++	+++	+++
Dry	100°C.	144	++	+	+
Dry	120°C.	24	++	—	++
Dry	120°C.	144	—	—	—

BASAL DIETS USED IN ASSAYING FOODSTUFFS FOR VITAMINS B, G AND B_4

In view of these results we have modified the diets originally proposed for B and G studies. Autoclaving the ration as previously indicated (table 3) destroys some of the antiparalytic factor. To cover this deficiency, 2 per cent of whole liver substance is added. The composition of the diet (ration 242A) now used for B assay studies is as follows:

Yellow corn	57	Autoclaved 5 hours 15 pounds' pressure
Wheat middlings	25	
Crude casein	12	
NaCl	1	
$Ca_3(PO_4)_2$	1	
$CaCO_3$	1	
Special whole liver substance (Wilson)	2	
Cod liver oil	1	

The 2 per cent $CaCO_3$ has been changed to 1 per cent $CaCO_3$ and 1 per cent $Ca_3(PO_4)_2$ and the cod liver oil reduced from 2 to 1 per cent to make this part of the ration comparable to the method for vitamin D assay in vitamin D carriers for poultry by Hart, Kline and Keenan ('31).

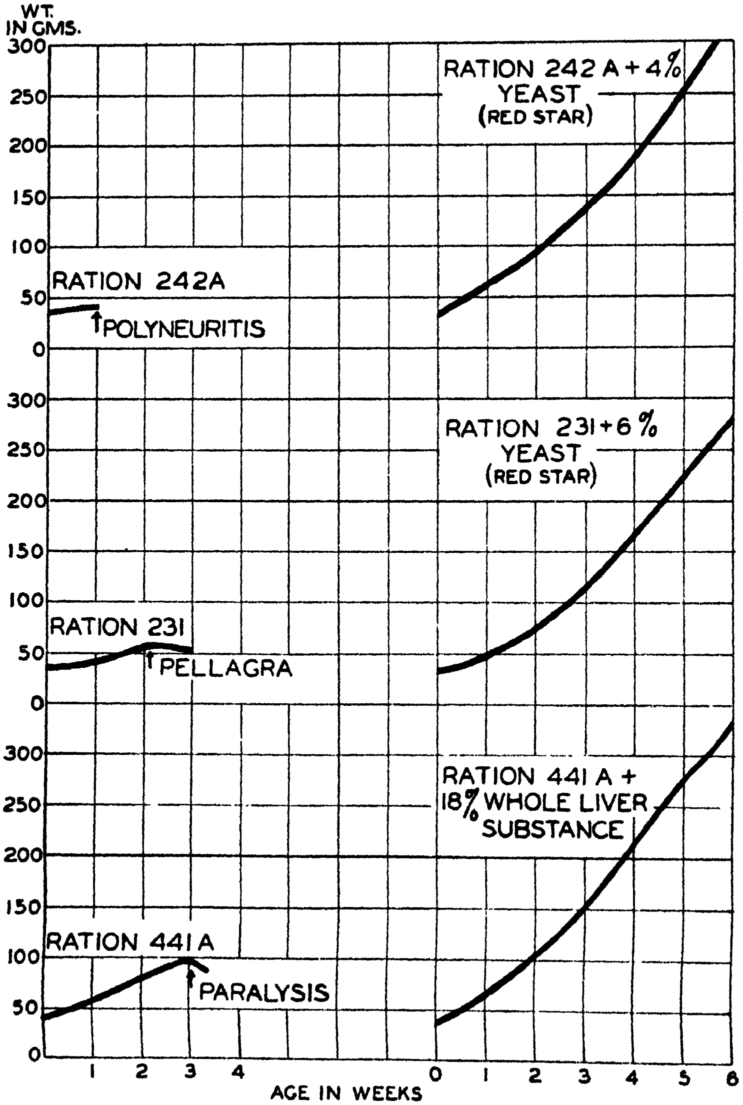

Figure 1

Typical growth curves of chicks confined to this basal diet only and with 4 per cent yeast addition are given in figure 1.

The inadequacy of the basal grain diet for vitamin G studies, which has been heated to 100°C. for 144 hours and the superiority of the ration treated at 120°C. for 24 hours has been discussed. Not only does this diet contain more vitamin B_4, but it is more deficient in the antipellagric factor than the diet previously proposed. The composition of the basal diet now used for G assay studies (ration 231) is

Yellow corn	59	Heated 120°C. for 24 hours
Wheat middlings	25	
Crude casein	12	
NaCl	1	
$CaCO_3$	1	
$Ca_3(PO_4)_2$	1	
Cod liver oil	1	

The performance of birds confined to this diet alone and with a 6 per cent yeast supplement is given in figure 1.

The basal vitamin B_4 deficient diet used for studies on this factor is the same as that previously described with the exception that the casein is autoclaved. It has been designated ration no. 441A and has the composition—

Dextrin	53.5
Autoclaved casein	24.0
Salts	2.5
Yeast (Anheuser Busch)	8.0
Autoclaved liver residue	10.0
Cod liver oil	2.0

The incidence of the abnormality in chicks confined to this diet is very uniform. The behavior of birds confined to the basal diet no. 441A and with 18 per cent whole liver is given in figure 1.

DISCUSSION

As a result of the knowledge gained from a supply of the stability of vitamins B, G and B_4, basal diets have been formulated which may be used in testing the distribution of these factors in foods. Although the heat treatments utilized in these studies are not comparable, in general, with the methods used in the process of preservation and preparation of human foods, they are, however, quite similar to methods used by commercial companies in the preparation of animal foods.

These studies emphasize the relative lability of vitamin B_4. This fact, combined with the limited supply of this component in human dietaries substantiates the contention, previously made, "that at least this factor may be a critical one in normal nutrition."

Our knowledge concerning the chemistry of the vitamin B complex has increased considerably during the past 3 years. Windaus, Tschesche and Ruhkopf ('32), Ohdake ('34), and others have shown that their antineuritic concentrates contain sulphur in the molecule. It is possible that the presence of this element renders the molecule more labile. That the inactivation may be one of hydrolysis is suggested from the fact that degradation is catalyzed by the presence of moisture in the heating medium.

Kuhn, György and Wagner-Jauregg ('33) have correlated vitamin G activity with the flavine pigments obtainable from milk, eggs, or liver and bearing the empirical formula $C_{17}H_{20}O_6N_4$. It is difficult to visualize what happens to the pigment when such foodstuffs as mentioned above are subjected to treatments which will render them deficient in this factor. It is our opinion that further work is necessary to substantiate the view that the antipellagric factor is an integral part of the pigment molecule.

With the exception of Tschesche's ('33) report that Reader's vitamin B_4 concentrate (Barnes, O'Brien and Reader, '32), was a substance very closely allied with adenine, little is known concerning the chemistry of this factor. Barnes, O'Brien and Reader ('32) have prepared concentrates of this factor by methods which are somewhat similar to the methods used for the preparation of vitamin B, with one exception, that of differential adsorption upon charcoal. Vitamin B is adsorbed at a pH of 7 whereas vitamin B_4 is selected preferentially by the adsorbent at a pH of 1. This demonstrates the proximity in chemical characteristics of vitamins B and B_4. The results presented in this paper demonstrate the similarity in heat stability of the antineuritic and antiparalytic components. We suggest, therefore, that the molecule of vitamin B is possibly very similar to that of vitamin B_4 and the antiparalytic factor may be inactivated by the same process which functions in the degradation of vitamin B.

Although autoclaving the grain diet inactivates the greater portion of the vitamin B, vitamin B_4 is comparatively stable. Autoclaving liver inactivates both the antineuritic and antiparalytic factors. This emphasizes the influence of surrounding or accompanying material on the rate of B_4 destruction. The similarity in heat stability of vitamins B and B_4 in yeast and liver raises the question as to whether or not some of the abnormalities which have been ascribed to the polyneuritic syndrome may not be due in part at least to a deficiency of vitamin B_4.

CONCLUSIONS

1. Vitamin B(B_1) in yeast, liver, and in a natural grain ration was destroyed completely by autoclaving and inactivated to a great extent in yeast and liver by heating in the fresh state at 100°C. for 24 hours.

2. Vitamin G(B_2) may be inactivated in the foodstuffs tested by a dry heat treatment of 120°C. maintained 24 hours. Vitamin B is relatively stable to this thermal treatment. Under such conditions vitamin G does not conform to the conception that it is the more heat stable factor.

3. Vitamin B_4 is similar in heat stability to that of vitamin B. This suggests that much of the past work with vitamin B deficiency may be due, in part, to a deficiency of B_4.

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A STUDY OF THE IRON METABOLISM OF NORMAL WOMEN[1]

MARGARET A. OHLSON[2] AND KATE DAUM
Department of Nutrition, Graduate College, State University of Iowa

ONE FIGURE

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On the basis of iron balance studies completed before 1907, Sherman ('07) proposed our present dietary standard of an average of 15 mg. of iron daily for the normal adult. The subjects of these balance studies were men who excreted an amount of iron ranging from 7 to 16 mg. when fasting or confined to relatively restricted dietaries, such as bread and milk or bread and egg white. Since 1907, more satisfactory methods for the analysis of iron have been developed and a large amount of data is now available concerning the iron content of common foods and the influence of manufacture, preservation and cookery. When considering the sources of iron in the usual mixed dietary, these gains and losses of iron in the preparation of food and certain trends in food selection may be significant in determining the adequacy of the diet both from the standpoint of the total iron ingested and the ability of the gastro-intestinal tract to utilize the material provided. Of the iron rich foods, meat, eggs, nuts and whole grain cereals are also protein rich foods. With the present trend to reduce the amount of meat and whole grain cereals ingested (Bogert,

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[1] A complete record of diets, experimental method and data on balances is on file at the library of the University of Iowa, Iowa City, Iowa.

[2] A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Department of Nutrition in the Graduate College of the State University of Iowa, June, 1934.

'31), green vegetables have been suggested as a means of balancing the iron intake. While such a balance is theoretically possible, the absolute value of green vegetables to the human organism is yet to be established. The recent review of Schultz et al. ('33) makes further discussion of this point unnecessary, except to emphasize the fact that the addition of spinach to the formula of one of his infants caused an iron excretion in excess of that supplied by the vegetable. In her study of the food intake of pregnancy, Coons ('32) reported that the least satisfactory iron retentions were found in the one subject whose diet included the greatest amount of vegetables and commented on an increased dry weight of stool during one particularly unfavorable period. Sherman ('07) suggested that slight gastro-intestinal variations might influence markedly the excretion of iron, and we find in the clinical literature (Alvarez, '31) the warning that the individual tolerance for vegetables by the human gastro-intestinal tract may be quite variable.

Of recent iron balance studies on normal individuals, the papers of Schultz, Morse and Oldham ('33), Rose et al ('30) and of Leichsenring and Flor ('32) deal with the iron exchange of infants and preschool children under the carefully controlled food conditions characteristic of those years. Reznikoff et al. ('34) have reported the iron exchange of a normal man during 7 months of hospitalization. In this time, their subject was repeatedly in iron balance on an intake of 10 mg. However, the increase of dietary iron to 26 mg. and the use of parenteral liver therapy and later of inorganic iron resulted in a large total retention and a 25 per cent increase in hemoglobin during the 7 months of observation.

This study of the iron exchange of three normal women under essentially unrestricted conditions of living and diet was undertaken since; so far as the writer is aware, no such study has been reported recently.

EXPERIMENTAL

Only those factors which have a particular bearing upon the interpretation of the experimental data are mentioned.

The subjects studied were three healthy young women who were carrying an active schedule of laboratory work. The metabolic periods were carried out at intervals during a total of 2½ months. Periods during which complete collections were made ranged from 5 to 15 days and included at least one menstrual period for each subject. Menstruation was regular and normal in all cases, the periods of 4 to 5 days' duration at 28- to 30-day intervals, and the flow was average in amount. A total of 56 experimental days was recorded.

Each subject was consulted concerning her food likes and the approximate amount of food which she customarily ate. This information was translated into a foundation dietary which was varied in the choice of meat, vegetables and desserts. Calories were adjusted from time to time by slight variations in the bread, butter and sugar consumed. An amount of food needed for 2 or 3 days was secured at one time and stored under uniform conditions. Meals were prepared in a small kitchen resembling a household unit in its equipment of utensils. At the time the meal was served, an equivalent portion of each food was set aside for analysis and was carefully stored in Pyrex glassware. All subjects drank measured amounts of tap water from the same source and an equivalent portion was added to the total food sample. The protein and caloric adequacy of the diets was checked by means of nitrogen balances and frequent records of body weight.

All collections of food and excreta were made directly into glass containers which were covered with glass plates and were prepared for analysis without transfer. Analyzed carmen was used for marking the beginning and end of each period. During the menstrual periods, a standard brand of cellulose pad was used and a sufficient supply for the entire study was purchased in a single lot and sampled at random for blank analysis.

Sulfuric acid digests of food and feces were prepared by the method of Stearns ('29). All mineral analyses of excreta were carried out on a sulfuric acid ash prepared in 6-inch

Pyrex test tubes on a sand bath. The modification of the permanganate titration method described by Murray ('24) was adopted for the measurement of iron. A sample containing about 0.15 mg. of iron was used for analysis except in the case of urine. Copper was determined by the method of Ansbacher, Remington and Culp ('31).

TABLE 1

Recovery data

MATERIAL	AMOUNT OF SAMPLE	ADDED	IN SAMPLE	FOUND	PER CENT RECOVERY
			Iron		
	cc.	*mg.*	*mg.*	*mg.*	
Urine	25	0.25	0.014	0.244	92.0
	15	0.25	0.002	0.258	102.4
	10	0.25	0.005	0.245	96.0
	10	0.25	0.007	0.249	96.8
Feces digest	½	0.25	0.120	0.364	97.6
	½	0.25	0.148	0.385	94.8
	½	0.25	0.108	0.346	95.2
	½	0.25	0.111	0.367	102.4
Digest of menstrual pads	1	0.25	0.113	0.358	98.0
			Copper		
Feces digest	1	0.003	0.006	0.009	100.0
	1	0.01	0.018	0.027	90.0
Food digest	5	0.01	0.006	0.015	90.0
	5	0.01	0.006	0.015	90.0

DISCUSSION OF EXPERIMENTAL RESULTS

The difficulties involved in the micro-analysis of minerals need no comment. The range of error of the iron method used was from 0 to 14 per cent with an average of 5.6 per cent. It was possible to check the copper content of duplicate samples of ash to within 13 per cent with an average of 5 per cent. Recovery data for both copper and iron are given in table 1.

Owing to the large amount of free fat present, two food samples of period 1 were lost because of 'bumping' of the wet digest. These diets were calculated from standard food tables (Waller, '31, and Hodges and Peterson, '31). The comparison of determined and similarly calculated values for all dietaries indicated that the error introduced by this procedure would be within 10 per cent.

The menus chosen are summarized in table 2. The food ingested was generous in amount and variety and, it is believed, characteristic of the food habits of these subjects. Certain trends in food selection were noted: there was no cereal used, except as white bread and pastry; at least four glasses of milk were taken daily and ice cream was the choice for dessert; except in one diet where eggs were substituted, meat was used daily; sugar, candy and preserves were eaten

TABLE 2

Summary of diets

PERIOD	DIET	TOTAL FOOD PORTIONS IN GRAMS											
		Milk, cream, ice-cream	Butter	White bread	Meat	Potatoes	Citrous fruit [1]	Other fruit	Tomatoes [2]	Vegetables	Candy, sugar	Sandwich spread [3]	Pastry
1	1	975	45	100	110	100	165	75	250	190	70	24	0
	2	950	45	100	110	100	165	80	250	130	60	37	0
	3	950	45	70	150	100	275	0	250	173	40	26	0
	4	980	30	70	150	100	265	70	250	125	70	30	0
	5	980	40	80	160	100	165	180	250	105	45	40	0
2	1	925	40	100	00[4]	100	265	30	250	125	45	140	0
	2	945	40	100	45[5]	100	245	30	250	125	50	40	0
	3	945	45	100	115	100	245	30	250	125	50	40	0
	4	915	45	100	140	100	265	30	250	125	45	40	0
3	1	975	55	100	90	100	175	100	225	220	75	15	55
	2	975	55	100	100	100	175	100	250	210	75	15	45
	3[6]	975	55	100	120	100	175	100	250	210	80	15	25
	4[6]	975	40	70	120	100	175	100	250	150	105	15	100
	5[6]	975	40	70	120	100	175	100	250	210	120	15	45

[1] Includes a daily portion of 150 cc. of orange juice.

[2] Tomato juice, except in the third period when 100 gm. of fresh tomatoes were substituted for an equivalent amount of juice.

[3] Choice of egg, bacon, peanut butter, etc.

[4] Eggs were substituted.

[5] Cold roast meat.

[6] Subject C was served 0.9 of each food.

freely; daily portions of at least two servings of fruit and two servings of green vegetable in addition to potatoes and tomato juice were ingested. The green vegetable allowance was further increased during the third period by the addition of 100 gm. of freshly cooked green beans to each diet, except the fourth. The range of iron values for the total diets was from 11.26 to 15.57 mg. with an average of 13.78 mg. Milk furnished 14 to 16 per cent of the dietary iron, meat about 35 per cent and vegetables from 30 to 35 per cent.

The agreement between the calculated and determined values for food iron was of interest, since the method of collection of the food and the use of tap water for drinking approximated the procedure of preparing the average dietary. Determined values for iron of the diets as ingested were equal to or above the calculated value in three diets with an average increase of 3 per cent, and below the calculated values in eleven diets with an average decrease of 9 per cent. Rose et al. ('30), and Leichsenring and Flor ('32) found only 70 per cent of the calculated amounts of iron in their dietaries, but they controlled all contamination during preparation and used only distilled water for drinking purposes. From these figures, it would seem that dietary estimates of the iron intake based on the data of available food tables would yield satisfactory results for meal planning in any situation other than the strict metabolic study.

Complete daily and period balances are summarized in table 3. In studying the iron excretions, only the fecal and menstrual iron losses were significant in determining the iron balance, although iron was found in all except one of the urine samples. The average daily output of iron in all urines was 0.52 mg. When the menstrual specimens which contained blood were excluded, an average figure of 0.38 mg. was obtained. Subject A seemed to show a tendency to excrete greater amounts of urinary iron than subjects B and C. Coons ('32) commented on a similar individual trend in certain of of her cases.

Since fecal iron was determined daily, the curve of excretion was very irregular. However, a parallelism of fetal nitrogen and fetal iron was noted particularly on the days of maximum and minimum excretion (fig. 1). Coons ('32) and Reznikoff ('34) observed a relationship between iron output and dry weight of stool. The total fecal losses of iron, however, were excessive when slight gastro-intestinal discomfort and increased elimination followed the ingestion of string beans (subject A).

Subject A was in positive iron balance during the first two periods, but lost 51 mg. of iron during the third and longest period in which she was studied. During the 6 days of period 1, this subject stored 14 mg. of iron. This amount was equal to about 75 per cent of the 18 mg. lost in menstruation, which occurred on the first 4 days of the period. In period 2, which represented the inter-menstrual phase of the same monthly cycle, she was still retaining an average of 0.44 mg. of iron daily. Period 3 shows an average daily loss of iron of 3.43 mg. There are two factors to be considered in interpreting this finding: first, menstruation which occurred from the second to the fifth day, inclusive, and, second, the addition of an extra portion of fresh green vegetable to the diet for the first 9 and the last 3 days of the period. An average daily increase of fecal nitrogen of 0.17 gm. occurred in this period (fig. 1). After the first few days, the subject complained of gas and general discomfort and attributed the difficulty to the added vegetable. The green beans were discontinued for 3 days and the fecal nitrogen fell on the third day to below the level of periods 1 and 2, but rose when the vegetable was included again. For the first 5 days of this period, which included menstruation, the average iron balance was positive with a daily average storage of 3.61 mg. However, from this point the fecal iron rose, reaching a peak of 32.48 mg. on the eighth day, and 29.79 and 28.37 mg. on the tenth and eleventh days, respectively. The average daily amount ingested was 13.76 mg. After discontinuing the string beans, a positive balance averaging 1.98 mg. was found for the twelfth, thir-

TABLE 3

Summary of balance studies

SUBJECT	PERIOD	DIET	NITROGEN			IRON			COPPER			COMMENT
			Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
			gm.	*gm.*		*mg.*	*mg.*		*mg.*	*mg.*		
A	1	1	11.99[1]	10.77	+ 1.22	14.41[1]	1.29	+ 13.12	0.97[1]	0.00	+ 0.97	Menstruation (some material lost on clothing)
		1	11.99[1]	12.77	— 0.78	14.41[1]	21.38	— 6.97	0.97[1]	2.90	— 1.73	
		2	13.10	10.71	+ 2.49	13.64	1.06	+ 12.58	0.89	0.00	+ 0.89	
		2	13.10	13.10	0.00	13.64	23.90	— 10.26	0.89	2.60	— 1.71	
		3	12.72[1]	10.90	+ 1.82	13.66[1]	1.25	+ 12.41	1.03[1]	0.00	+ 1.03	
		3	12.72[1]	10.41	+ 2.31	13.66[1]	20.20	— 6.54	1.03[1]	2.69	— 1.66	
	Averages											
	Entire period		12.60	11.44	+ 1.16	13.90	11.51	+ 2.39	0.96	1.37	— 0.40	
	Menstruation 4 days		12.55	11.84	+ 0.71	14.03	11.97	+ 2.12	0.93	1.38	— 0.45	
	Post menstruation 2 days		12.72	10.66	+ 2.06	13.66	10.73	+ 2.94	1.03	1.35	— 0.22	
A	2	2	10.81	11.26	— 0.45	11.26	22.17	— 10.91	0.93	2.09	— 1.16	
		2	10.81	11.84	— 1.03	11.26	6.84	+ 4.42	0.93	0.86	+ 0.07	
		3	12.17	12.31	— 0.14	13.90	19.77	— 5.87	1.12	1.49	— 0.37	
		3	12.17	13.39	— 1.22	13.90	8.73	+ 5.17	1.12	0.93	+ 0.19	
		3	12.17	10.82	+ 1.35	13.90	4.49	+ 9.41	1.12	0.75	+ 0.37	
	Averages											
	Inter mestruation. Total		11.63	11.92	— 0.29	12.84	12.40	+ 0.44	1.04	1.22	— 0.18	
	Diet 2, 2 days		10.81	11.55	— 0.74	11.26	14.51	— 3.25	0.93	1.88	— 0.55	
	Diet 3, 3 days		12.17	12.17	0.00	13.90	11.00	+ 2.90	1.12	1.06	+ 0.06	
A	3	1	11.05	8.82	+ 2.23	13.54	0.24	+ 13.30	1.16	0.00	+ 1.16	Menstruation
		1	11.05	10.20	+ 0.85	13.54	20.82	— 7.28	1.16	0.91	— 0.75	
		1	11.05	10.29	+ 0.76	13.54	22.45	— 8.91	1.16	1.80	— 0.64	
		2	10.98	9.65	+ 1.33	15.36	9.52	+ 5.84	1.35	1.06	+ 0.29	
		2	10.98	9.16	+ 1.82	15.36	0.27	+ 15.09	1.35	0.00	+ 1.35	
		2	10.98	12.92	— 1.94	15.36	28.17	— 12.81	1.35	2.14	— 0.79	
		3	11.63	10.25	+ 1.38	13.74	9.23	+ 4.51	1.11	0.93	+ 0.18	

		3	11.63	12.69	— 1.06	13.74	32.48	— 18.74	1.11	1.67	— 0.56	
		3	11.63	10.27	+ 1.36	13.74	17.83	— 4.09	1.11	1.68	— 0.57	
		4	11.00	11.33	— 0.33	12.49	29.79	— 17.30	1.32	1.43	— 0.11	
		4	11.00	10.10	+ 0.90	12.49	28.37	— 16.07	1.32	0.84	+ 0.48	
		4	11.00	10.40	+ 0.60	12.49	18.40	— 5.91	1.32	1.46	— 0.14	
		5	10.83	9.10	+ 1.73	13.65	0.31	+ 13.34	0.80	0.00	+ 0.80	
		5	10.83	11.55	— 0.72	13.65	15.13	— 1.48	0.80	1.85	— 1.05	
		5	10.83	10.80	+ 0.03	13.65	24.53	— 10.88	0.80	1.63	— 0.83	
	Averages											
	Total		11.10	10.50	+ 0.60	13.76	17.18	— 3.43	1.15	1.23	— 0.08	
	Pre-menstruation 1 day		11.05	8.82	+ 2.23	13.54	0.24	+ 13.30	1.16	0.00	+ 1.16	
	Menstruation 4 days		11.02	9.83	+ 1.19	14.45	13.27	+ 1.19	1.26	1.19	+ 0.07	
	Post menstruation 4 days		11.47	11.53	— 0.06	14.15	21.13	— 7.78	1.17	1.61	— 0.44	
	3 days		11.00	10.61	+ 0.39	12.49	25.58	— 13.09	1.32	1.24	+ 0.08	
	3 days		10.83	10.48	+ 0.35	13.65	13.32	+ 0.33	0.80	1.16	— 0.36	
B	2	1	10.05	11.26	— 1.21	12.62	20.93	— 8.31	0.86	1.87	— 1.01	Menstruation
		1	10.05	9.05	+ 1.00	12.62	17.96	— 5.34	0.86	0.61	+ 0.25	
		1	10.05	9.42	+ 0.63	12.62	18.97	— 6.35	0.86	0.92	— 0.06	
		2	10.81	9.42	+ 1.39	11.26	13.03	— 1.77	0.93	1.32	— 0.39	
		2	10.81	10.54	+ 0.27	11.26	19.31	— 8.05	0.93	1.23	— 0.30	
		2	10.81	12.55	— 1.74	11.26	19.47	— 8.21	0.93	1.20	— 0.27	
		3	12.17	11.60	+ 0.57	13.90	8.09	+ 5.81	1.12	0.66	+ 0.46	
		3	12.17	11.63	+ 0.54	13.90	8.23	+ 5.67	1.12	1.40	— 0.28	
		3	12.17	12.41	— 0.24	13.90	20.32	— 6.42	1.12	1.78	— 0.66	
		4	12.04	11.45	+ 0.59	15.57	14.26	+ 1.31	1.12	0.48	+ 0.64	
		4	12.04	12.94	— 0.90	15.57	12.03	+ 3.54[2]	1.12	1.20	— 0.08	
		4	12.04	11.95	+ 0.09	15.57	7.04	+ 8.53	1.12	0.84	+ 0.28	
	Averages											
	Total 12 days		11.27	11.16	+ 0.11	13.34	15.00	— 1.67	1.01	1.13	— 0.12	
	Pre-menstruation 1 day		10.05	11.26	— 1.21	12.62	20.93	— 8.31	0.86	1.87	— 1.01	
	Menstruation 4 days		10.43	9.61	+ 0.82	11.94	17.42	— 5.48	0.90	1.02	— 0.12	
	Post menstruation 4 days		11.83	12.02	— 0.19	13.24	14.03	— 0.79	1.07	1.26	—.0.19	
	3 days		12.04	12.11	— 0.07	15.57	11.11	+ 4.46	1.12	0.84	+ 0.28	

TABLE 3—*Continued*

SUBJECT	PERIOD	DIET	NITROGEN			IRON			COPPER			COMMENT
			Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
			gm	*gm.*		*mg.*	*mg.*		*mg.*	*mg.*		
C	1	2	13.10	11.18	+ 1.92	13.64	18.56	— 4.92				**Menstruation**
		3	12.72[1]	10.39	+ 2.33	13.66[1]	12.19	+ 1.47				
		3	12.72[1]	9.98	+ 2.74	13.66[1]	12.08	+ 1.58				
		4	11.96	12.55	— 0.59	13.60	13.27	+ 0.33				
		4	11.96	12.13	— 0.17	13.60	16.01	— 2.41				
		5	13.40	13.83	— 0.43	15.47	17.70	— 2.22				
		5	13.40	13.29	+ 0.11	15.47	15.75	— 0.27				
	Averages											
	Total		12.75	11.91	+ 0.84	14.16	15.08	— 0.90				
	Menstruation 5 days		12.49	11.13	+ 1.36	13.63	14.42	— 0.59				
	Post menstruation		13.40	13.56	— 0.16	15.48	16.73	— 1.25				
C	3	2	10.98	10.02	+ 0.96	15.36	19.55	— 4.19	1.35	1.09	+ 0.24	
		2	10.98	11.05	— 0.07	15.36	11.90	+ 3.46	1.35	1.23	+ 0.12	
		3[3]	10.47	7.92	+ 2.55	12.37	15.16	— 2.79	1.11	0.79	+ 0.32	
		3	10.47	10.04	+ 0.43	12.37	10.53	+ 1.84	1.11	0.84	+ 0.27	
		3	10.47	9.61	+ 0.86	12.37	13.27	— 0.90	1.11	0.98	+ 0.15	
		4[2]	9.90	9.79	+ 0.11	11.24	19.60	— 8.36	1.32	2.27	— 0.95	
		4	9.90	8.69	+ 1.21	11.24	10.67	+ 0.57	1.32	0.98	— 0.34	
		4	9.90	7.86	+ 2.04	11.24	11.64	— 0.40	1.32	0.59	+ 0.73	
		5[3]	9.75	9.07	+ 0.68	12.28	12.65	— 0.37	0.80	1.50	+ 0.70	
		5	9.75	9.58	+ 0.17	12.28	22.52	— 10.24	0.80	1.30	— 0.50	
		5	9.75	10.28	— 0.53	12.28	16.02	— 3.74	0.80	1.06	— 0.26	
	Averages											
	Total		10.21	9.45	+0.76	12.58	14.87	— 2.29	1.13	1.14	— 0.02	
	Dietary Periods 1, 2 days		10.98	10.54	+ 0.44	15.36	15.73	— 0.37	1.35	1.16	+ 0.19	
	2, 3 days		10.47	9.19	+ 1.28	12.37	12.99	— 0.62	1.11	0.87	+ 0.24	
	3, 3 days		9.90	8.78	+ 1.12	11.24	13.98	— 2.74	1.32	1.28	+0.04	
	4, 3 days		9.75	9.64	+ 0.11	12.28	17.06	— 4.78	0.80	1.29	— 0.49	

[1] Calculated figures.

[2] Urine figure omitted because of contaminated specimen.

[3] Subject C ingested exactly 0.9 of the dietary as outlined.

teenth and fourteenth days, but the effect of including the vegetable again was evident on the last day when the retention fell to a negative 10.88 mg.

Subject C lost a total daily average of 2.29 mg. of iron during the third period. This subject was in slightly negative iron balance during the first period also. In addition to the 25.68 mg. of iron lost in menstruation, her total urine and fecal output averaged 15.08 mg. on a daily intake of 14.16 mg.

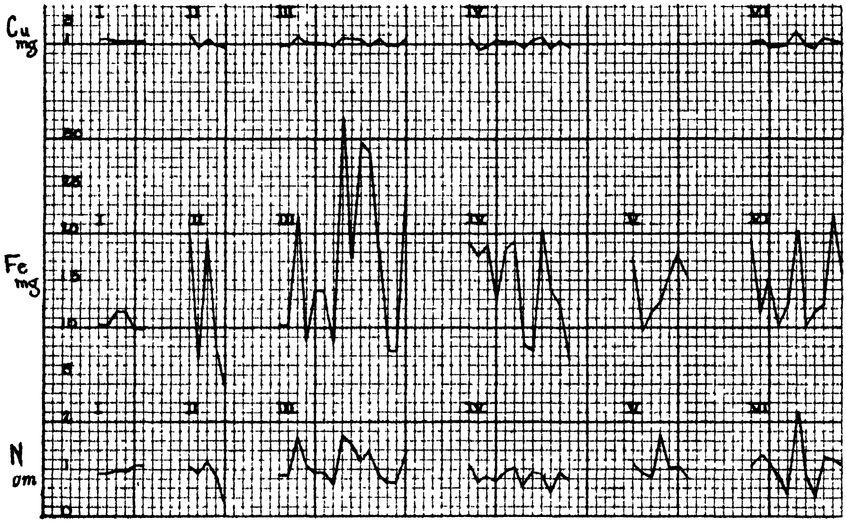

Fig. 1 Comparison of fecal copper, iron and nitrogen.

I, Subject A, period 1
II, Subject A, period 2
III, Subject A, period 3
IV, Subject B, period 2
I, Subject C, period 1
VI, Subject C, period 3

This subject had had the least experience in metabolic studies and just what part emotional adjustment played in determining her gastro-intestinal efficiency is speculative.

The findings on subject B during the single period of observation offer an interesting contrast to the periods for subject A. Subject B was in strikingly negative iron balance during menstruation since she lost an average of 6.34 mg. of iron for each of 6 days. The next 3-day period was marked by a positive balance of 1.35 mg. per day and this balance in the last 3 days of the period increased to an average of 4.46 mg. The parallelism between fecal nitrogen and fecal iron was again striking. Although the total iron balance of this subject for the period was negative to the extent of a daily loss of 1.67 mg., the rate of storage during the last 6 days indicated that rapid compensation was taking place. This subject lost 41.90 mg. of iron in the menstrual hemorrhage which is the largest amount recorded in this series.

The complete data for the losses of menstruation are summarized in table 4. With the exception of subject B, the menstrual losses were all smaller than the 34, 38 and 42 mg. of iron reported by Gillett et al. ('18) for three normal women. Although these reported iron losses are not large, they will result in a deficit if repeated at regular intervals unless preceded or followed by a storage of iron. Such a deficit is suggested by the hypochromic anemia of women described by Bloomfield ('32), who found a history of excessive blood losses in only a small proportion of cases. Mettier ('31) reported a series of hypochromic anemia cases where a deficient dietary consumed over a period of years appeared to be an etiological factor. The nitrogen losses of the menstrual hemorrhage were also low as compared with the range of from 0.656 to 1.92 gm. found by Sherman, Gillett and Pope ('18).

TABLE 4

Analysis of menstrual loss

SUBJECT	PERIOD	NITROGEN	IRON	COPPER	FE : N	CU : FE
		gm.	*mg.*	*mg.*		
A	1	0.64	18.17	0.39	1: 35	1: 46
A	3	0.89	32.35	0.61	1: 28	1: 53
B	2	0.46	41.92	0.19	1: 11	1: 221
C	1	0.80	25.68	0.24	1: 31	1: 107

Dietary copper as determined ranged from 0.80 to 1.39 mg. per day. The calculated copper value for the ten diets ranged from 6 to 37 per cent higher than the determined values. The copper to iron ratio of the dietaries was from 1 to 12 to 1 to 17.

Copper was found in traces in twenty-four out of twenty-seven urine samples which were analyzed, but the amount was in no case sufficient for calculation. This was somewhat amazing in view of the findings of Rabinowitch ('33), who reported a daily urinary copper ranging from a trace to 0.70 mg. An examination of the copper in the stool, however, indicated that the amounts ingested could be found in the fecal output. Negative copper balances ranging from a daily average of 0.02 to 0.40 mg. were observed. In all cases, except subject A in periods 1 and 2, the average amount of copper lost was within 10 per cent of the intake, which is perhaps also within the limitations of the method. In period 1, subject A lost a daily average of 0.40 mg. and in period 2 a daily negative retention of 0.18 mg. was observed. The subject was storing iron during both of these periods. There appeared to be some tendency for high excretions of copper to parallel high fecal iron, but this parallelism was not entirely consistent.

It would seem unwise to generalize from the few observations presented here. There seemed to be an extremely labile mechanism regulating the iron balance and that this mechanism was closely related to intestinal function was obvious. These normal subjects were apparently able to compensate for temporary intestinal inefficiency with marked iron retentions when the irritating situation was relieved. It would be reasonable to suppose, however, that repeated gastro-intestinal irregularities might exhaust the iron reserves, particularly if the intake of food were limited. Although the average iron content of these diets did not quite reach the amount suggested by Sherman ('07) as an optimal intake, the diets as a whole were generous in all known food constituents. Sherman ('07) has correlated a lack of calcium with negative iron balances. The calcium intake of this study was in excess of a gram per day. The part which vegetable fiber played in determining the response to string beans is open to conjecture. Adolph and Wu ('34) did not find an increased acceleration of the passage of food through the tract or an increase in fecal nitrogen when cabbage fiber was fed at high levels. If fruit and fruit juices are included in the total, the vegetable intake of all the diets used in this study was between 500 and 700 gm., but almost half of the amount was in the form of strained fruit juices so that the fiber content of the diets was not raised proportionately.

SUMMARY

1. Iron balances are presented on three normal women for six periods totaling 56 experimental days. On self-chosen diets containing liberal amounts of protein, calories and protective elements, the average daily amount of iron ingested was 13.78 mg. The average daily iron excretion for all periods was 14.95 mg.

2. Two periods of positive iron balance were recorded for subject A. Subjects B and C were both in negative iron balance as was subject A for the third period of study. Negative iron balances were associated with increased fecal nitrogen. No relationship was observed between iron retention and the iron losses of menstruation or between the balance of nitrogen or copper and the balance of iron.

3. Totals of 18.16, 25.68, 32.35 and 41.90 mg. of iron, respectively, were lost in the menstrual hemorrhage during each of four periods.

4. The copper to iron ratios of the ingested food ranged from 1 to 12 to 1 to 17. Copper was excreted through the stool.

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A STUDY OF THE PROTEIN NEEDS OF PRESCHOOL CHILDREN

AMY L. DANIELS, MARY K. HUTTON, ELIZABETH M. KNOTT, OLIVE E. WRIGHT, GLADYS J. EVERSON AND FLORENCE SCOULAR
Iowa Child Welfare Research Station, State University of Iowa, Iowa City

THREE CHARTS

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There is little information in the literature relative to the amount of protein which children of various ages beyond infancy should be given. From 10 to 12 per cent of the total calories, or approximately 1 gm. per pound of body weight is considered sufficient by most students of child nutrition, "provided the diet is of ample fuel value and the protein is of the right kind" (Sherman, '18). These standards have been arrived at more or less empirically, the results of observations of growth when the children were receiving 1 gm. or thereabouts per pound, and by a consideration of the percentage of the protein of human milk—a food presumably adapted to the needs of the child during its most rapidly growing period. "Practically, it is not possible to keep the protein as low as 10 per cent of the total calories and still observe the rule of a quart of milk per day. There seems to be no reason why conditions for growth need be less favorable when the protein calories constitute as much as 15 per cent of the total calories" (Rose, '27). Holt ('21) reports in a study of the food eaten by thirty-two children from 2 to 6 years of age in a children's home that an average of 3.5 gm. of protein per kilogram was taken daily; and an estimate of the protein eaten by normal healthy children of intelligent well-to-do families showed that the children between 2 and 3 years of age were taking 49 gm. or approximately 4 gm. per kilogram. Older children between 6 and 7 averaged 60 gm., or 3 gm. per kilogram. These data do not necessarily indicate child needs, but rather the amount contained in the foods eaten.

The most extensive study of the nitrogen metabolism of children is that of Wang, Hawks and Hays ('28) with both normal and underweight children between 5 and 13 years of age. Based on weights and observations of the activity of the children, these authors have grouped their findings into several categories: nitrogen metabolism of vigorous normal children; of children normal in weight only; of children from 5 to 10 per cent underweight, etc. The average nitrogen retentions of the various groups conformed rather consistently to these groupings, less being retained by the vigorous normal group and larger amounts by those who were below weight. For example, the average retention of the vigorous normal group reported was 0.041 gm. at an average ingestion of 0.448 gm. per kilogram, whereas the children who were normal in weight only retained an average of 0.088 gm. per kilogram at an ingestion of 0.516 gm. per kilogram. As the percentage of underweight increased, the average amount ingested as well as the amount retained increased. There were, however, quite wide variations among the individuals of different groups. These may be explained possibly by differences in the ages of the children or by variations in the amount of nitrogen ingested.

In a second study (Wang, Hawks and Kaucher, '28) retentions with both high and low ingestions were determined. In this as in the former study, the underweight children retained more than those of average weight whether on high or low protein diets. At an average ingestion of 0.537 gm. per kilogram by the normal children, more nitrogen was retained (0.075 gm. per kilogram) than in the previous study at the somewhat lower ingestion level (0.448 gm. per kilogram). With a much lower ingestion, 0.264 gm. per kilogram (corresponding to 0.75 gm. of protein per pound) an average of only 0.001 gm. per kilogram was retained. Several of the children at this low ingestion level were found to be in negative balance perhaps due to the fact that an insufficient amount of high grade protein was included in the diet.

That the protein requirement of children varies inversely with age and is directly proportional to the rate of growth is well known. In Wang's studies there was but one child below 8 years of age among the vigorous normal group (Wang, Hawks and Hays, '28). In the groups normal in weight only there are reported only five children 6 years of age or under who were up to standard weight. The nitrogen retentions of these varied from 0.026 to 0.172 gm. per kilogram on the low protein diet, whereas the retention of those slightly below average varied from 0.064 to 0.158 gm.; and although the ingestions also varied, with the exception of those on the very low ingestions, there appeared to be no significant correlation between the amount ingested and the amount retained. Altogether, there would seem to be too few cases from which to draw conclusions regarding the protein needs of the preschool child, a group just beyond babyhood which may need considerably more than the older child of whom Wang's group largely consisted.

Parsons ('30), in a series of nitrogen studies with diabetic children from 4 to 8 years of age, obtained positive balances with 0.5 gm. of protein per pound or 1.1 gm. per kilogram; similarly, Bartlett ('26), also studying diabetic children between 4 and 14 years of age, obtained positive nitrogen balances, growth in height, and gain in weight with normal activity at ingestion levels of 0.6 to 1.0 gm. of protein per kilogram. It will be noted that the amount of protein taken by these children was considerably less than that taken by the children reported by Holt, or than the generally accepted amount of 1 gm. per pound, lower even than the low levels used by Wang for children of similar ages with whom some negative balances were obtained. Are these low amounts used by Parsons and Bartlett sufficient, or are the higher amounts more nearly in accord with the child's optimum physiological performance? A positive nitrogen balance obviously cannot be the criterion by which to judge the protein needs of a given child. Growth is a prerogative of childhood. The rate of growth is limited by the inherent capacity of the individual and the available amount of any one of the various essential nutritive constituents between the minimum and the optimum; and although the children in both Parsons' and Bartlett's studies gained in weight and height, they may not have realized their inherent growth capacities. At any level of protein ingestion above that required to cover katabolism, a positive nitrogen balance would obtain, provided adequate protein were supplied. Furthermore, it seems probable that the nitrogen needs of children even at the same age differ, depending upon the inherent potentialities of growth. Not knowing this, enough protein should be given to cover possible needs. How much this is can be determined only by studies with a considerable number of children of various ages and at various levels of ingestion.

In the investigation herein reported, 121 nitrogen balance studies have been made with 24 normal children (15 boys and 9 girls) between 37 and 66 months of age. During the observation period, the children lived under controlled conditions from 14 to 21 days as described in previous reports from this laboratory (Daniels et al., '34). The caloric needs of the children were considered from the standpoint of both height and weight. With but few exceptions, food supplying 40 calories per inch, or between 80 and 112 calories per pound was given. During the study period, the children gained in weight, thus ruling out the possibility of undernutrition as a factor influencing the nitrogen retentions. The amount of protein given varied from 0.9 to 1.6 gm. per pound of body weight. This was divided evenly among the three meals. The actual amount fed necessarily differed somewhat from the estimated amount because of the fact that the food used varied slightly in composition from that of the published analyses and the children gained during the period; thus the final weights, the average of the daily weights used in calculations of ingestions and retentions, were often higher than the weights used when the diets were planned.

EXPERIMENTAL PROCEDURE

In the first part of the study, from 3 to 4 days were allowed for metabolic adjustments to the diets before the balance studies were made. In general, two periods of 5 days each exclusive of the preliminary adjustment periods constituted a first and a second metabolism period at a given nitrogen ingestion level. Because of the differences sometimes observed in the first and second successive metabolism periods, later in the study a longer period (from 5 to 7 days) was allowed for the metabolic adjustment, and in these the metabolism periods (called double periods) were extended to 10 days, thus ruling out possible errors which might accrue from incomplete fecal eliminations of a shorter period. Stools in all periods were marked with 0.2 gm. of carmine.

A change in the method of preparing certain of the foods also was made for these longer metabolism periods, partly for the purpose of facilitating the procedures and partly for greater accuracy. With the exception of the meat and eggs, all cooked foods were prepared at the beginning of the metabolism period, subsequently packed in sterile containers, and stored in a low temperature refrigerator. Meat for each serving was weighed at the beginning of the period, wrapped in waxed paper and kept frozen until ready for use. Distilled water only was used for cooking and drinking. All children received daily from 2 to 4 ounces of orange juice and 2 teaspoons of cod liver oil. Many of them received also four drops of Viosterol twice daily. This apparently was without influence on the nitrogen retentions.

Nitrogen determinations by the Kjeldahl method were made with aliquots of each of the 24-hour urinary excretions, whereas aliquots of the total dried fecal excretions of the entire period and aliquots of the food for a given day were used.

In the investigation, presumably only physically fit children were studied, that is, they were active, carried no elevated temperatures, exhibited no digestive disturbances, slept well and, with very few exceptions, ate well. Physical measurement standards for children of this age group are limited, the most extensive being those of the Iowa Child Welfare Research Station ('29). Comparing the weight for height of the children studied with these standards, it was found that the children in general compared favorably with these norms. A few, however, were above the average, while others were below. One child who was studied during ten periods remained about 11 per cent below his theoretical weight for height. Twenty-five per cent of the children came from a state orphanage, 25 per cent from a county juvenile home where the food conditions were exceptionally good, and 50 per cent from private homes.

In determining nitrogen retentions in terms of weight it is obvious that if a child is underweight, a higher retention value will be obtained than if this is determined in terms of normal weight. A similar situation with regard to ingestion prevails. Therefore, both ingestions and retentions in the children studied have been determined, 1) on the basis of actual weight, and 2) on the basis of theoretical weight. These have been grouped according to ingestions on a scale of five between 0.35 and 0.70 gm. per kilogram, first, second, and double metabolism periods being grouped separately.

RESULTS

With the exception of a few cases at the very high and low ingestion levels, higher nitrogen retentions were obtained during first metabolism periods, irrespective of the amount ingested, within the limits of the study. For example, with nitrogen ingestions between 0.55 and 0.59 gm. per kilogram, eight children who averaged slightly below (— 3.5 per cent) the normal weight for height retained 0.121 gm. per kilogram during the first metabolism periods, whereas six at the same ingestion level retained an average of 0.084 gm. during second metabolism periods (table 1). Similarly, seventeen children very slightly above the average weight (+ 1.4 per cent), receiving between 0.50 and 0.54 gm. per kilogram, retained 0.101 during first periods, and eleven, also slightly above (+ 1.1 per cent) average weight for height, retained 0.092 gm. during second successive periods.

TABLE 1

Relation between nitrogen ingestion and nitrogen retention based on actual weights of children

NITROGEN INGESTION PER KILOGRAM	NUMBER OF CASES	AVERAGE AGE	AVERAGE WEIGHT	NITROGEN RETENTION			PER CENT OVER + OR UNDER − AVERAGE WEIGHT	
				Average per kilogram	Standard deviation	Range per kilogram	Average	Range
First metabolism periods								
gm.		*mo.*	*kg.*	*gm.*	*gm.*	*gm.*		
0.70–0.74	0	—	—	—	—	—	—	—
0.65–0.69	3	65	16.3	0.114	—	0.111–0.117	— 7.9	—11.3, — 4.1
0.60–0.64	4	63	16.7	0.112	—	0.100–0.141	— 4.4	—12.5, + 5.0
0.55–0.59	8	53	15.4	0.121	0.017	0.096–0.153	— 3.5	—11.1, + 5.1
0.50–0.54	17	53	16.2	0.101	0.022	0.056–0.141	+ 1.4	— 9.8, + 9.5
0.45–0.49	15	57	16.9	0.083	0.021	0.054–0.117	+ 2.7	— 4.9, +15.4
0.40–0.44	11	58	18.1	0.066	0.009	0.047–0.077	+ 3.5	— 4.0, + 7.6
0.35–0.39	1	53	14.7	0.063	—	—	— 4.6	—
Second metabolism periods								
0.70–0.74	1	73	16.8	0.107	—	—	— 7.6	—
0.65–0.69	1	60	15.8	0.143	—	—	—11.3	—
0.60–0.64	2	61	17.4	0.101	—	0.083–0.122	+ 0.3	— 5.7, + 6.2
0.55–0.59	6	57	15.7	0.084	0.019	0.061–0.120	— 5.4	—12.0, + 3.2
0.50–0.54	11	53	15.9	0.092	0.019	0.060–0.119	+ 1.1	—12.3, + 9.1
0.45–0.49	13	61	18.0	0.078	0.02	0.044–0.117	+ 5.0	— 3.3, +11.6
0.40–0.44	6	61	18.9	0.068	—	0.053–0.084	+ 4.7	— 3.5, +11.5
0.35–0.39	0	—	—	—	—	—	—	—
Double metabolism periods								
0.70–0.74	0	—	—	—	—	—	—	—
0.65–0.69	1	54	15.9	0.099	—	—	— 4.9	—
0.60–0.64	3	61	16.9	0.097	—	0.091–0.110	— 4.3	— 7.9, + 2.6
0.55–0.59	7	51	16.5	0.075	0.013	0.054–0.092	— 0.7	— 8.0, + 8.5
0.50–0.54	2	61	16.8	0.086	—	0.077–0.095	— 1.3	— 4.1, + 1.5
0.45–0.49	0	—	—	—	—	—	—	—
0.40–0.44	8	62	17.1	0.069	0.013	0.052–0.096	+ 2.2	— 5.2, +16.0
0.35–0.39	1	40	14.4	0.055	—	—	— 0.4	—

With the exception of two cases at ingestions between 0.060 and 0.064 gm., retentions estimated on the theoretical weights showed similar relationships at the higher levels, that is, higher retentions during first metabolism periods, but somewhat closer agreement at the lower levels (table 2). With ingestions between 0.45 and 0.49 gm. per kilogram, the

TABLE 2

Relation between nitrogen ingestion and nitrogen retention based on theoretical weights of children

NITROGEN INGESTION PER KILOGRAM	NUMBER OF CASES	AVERAGE AGE	AVERAGE WEIGHT	NITROGEN RETENTION			PER CENT OVER + OR UNDER − AVERAGE WEIGHT	
				Average per kilogram	Standard deviation	Range per kilogram	Average	Range
				First metabolism periods				
gm.		*mo.*	*kg.*	*gm.*	*gm.*	*gm.*		
0.70–0.74	0	—	—	—	—	—	—	—
0.65–0.69	0	—	—	—	—	—	—	—
0.60–0.64	4	61	16.7	0.114	—	0.105–0.133	— 0.8	— 8.4, + 5.0
0.55–0.59	13	57	17.0	0.114	0.027	0.068–0.152	+ 2.4	—11.3, +12.5
0.50–0.54	16	55	16.4	0.102	0.018	0.061–0.123	+ 0.4	—12.5, +15.4
0.45–0.49	18	56	16.5	0.077	0.017	0.053–0.115	— 0.2	—13.4, + 7.6
0.40–0.44	7	56	16.5	0.071	0.014	0.064–0.097	+ 0.03	— 9.8, + 5.6
0.35–0.39	1	53	14.7	0.061	—	—	— 4.6	—
				Second metabolism periods				
0.70–0.74	0	—	—	—	—	—	—	—
0.65–0.69	2	68	18.2	0.092	—	0.084–0.099	— 0.7	— 7.6, + 6.2
0.60–0.64	2	59	16.7	0.126	—	0.124–0.129	— 4.0	—11.3, + 3.2
0.55–0.59	7	52	16.2	0.105	0.017	0.077–0.132	+ 2.6	— 5.7, +11.6
0.50–0.54	12	56	17.3	0.083	0.021	0.059–0.121	+ 3.6	—12, + 9.0
0.45–0.49	16	61	17.3	0.077	0.016	0.045–0.111	+ 0.5	—12.3, +11.5
0.40–0.44	1	65	16.7	0.059	—	—	— 3.5	—
0.35–0.39	0	—	—	—	—	—	—	—
				Double metabolism periods				
0.70–0.74	0	—	—	—	—	—	—	—
0.65–0.69	1	64	18.3	0.096	—	—	+ 2.6	—
0.60–0.64	2	54	17.3	0.090	—	0.087–0.094	+ 1.8	— 4.9, + 8.5
0.55–0.59	7	55	16.7	0.079	0.016	0.056–0.105	— 1.6	— 7.9, + 4.3
0.50–0.54	3	54	15.6	0.081	—	0.065–0.092	— 5.3	— 8.0, — 3.9
0.45–0.49	1	62	21.0	0.062	—	—	+16.6	—
0.40–0.44	6	59	16.5	0.073	—	0.054–0.098	— 0.7	— 5.2, + 4.5
0.35–0.39	2	56	15.7	0.056	—	0.054–0.059	— 2.2	— 4.1, — 0.4

average retentions based on actual weight during first and second periods were 0.083 and 0.078 gm., respectively, and 0.077 gm. during first and second periods when estimated on the theoretical weights. The closer agreement at these lower levels may be due to the fact that the children were more nearly physically fit as shown by the very slight variation from the theoretical weights, or there may have been too little protein in the diet to make possible the high retentions obtained at the higher ingestion levels. The range of retentions, however, would indicate that more nitrogen might have been retained by certain of the children had it been needed. With all groups above the minimum, the upper limit of retention was much higher than the lowest, or the average of the group.

High nitrogen retentions may not in all cases be due to previous nitrogen starvation, but rather to a greater need. The child destined to be tall will need more than the child who is inherently short. Retentions at ingestion levels of 0.40 to 0.44 gm. per kilogram suggest that possibly less than the optimum amount had been ingested at least for certain of the children studied, since low retentions were fairly consistent in both first and second runs, whether based on actual or theoretical weights, and the upper limit of retention is lower than those of the higher ingestion levels.

It should be noted that the retentions based on actual and theoretical weights at the various ingestion levels do not in all cases pertain to the same children, owing to the fact that estimations of ingestions based on theoretical weights sometimes placed certain of the children in a group above or below that estimated on actual weights. Moreover, the range of retentions at each ingestion level differed to a greater or less degree. The weight averages of the children of the various groups suggest that the high retentions may be due to the fact that the children were underweight, as suggested by Wang in explanation of certain high retentions among her groups. Therefore, in order to determine if these variations in retentions were due to weight variations of the children studied or to some other factor or factors, the retentions of a few of the children at various ingestion levels have been grouped according to their actual and theoretical weights together with the percentage of weight variation from their theoretical average (table 3).

These data indicate that weight alone is not concerned with these variations in retentions, since high retentions were found among children who were above average in weight as well as with those below average. For example, D.B. and D.P. were quite normal children and, although very slightly above the average weight, were well within the range of normality. At ingestions of 0.45 gm. per kilogram (theoretical weight) they retained 0.070 and 0.064 gm. per kilogram, respectively; whereas C.P., at approximately the same ingestion, retained 0.086 gm. per kilogram, and R.H., at a very slightly higher ingestion (0.48 gm. per kilogram of theoretical

TABLE 3

Comparison of nitrogen retentions of individual children in relation to actual and theoretical weight at various nitrogen ingestions

NAME	DATE	WEIGHT	BASED ON ACTUAL WEIGHT		BASED ON THEORETICAL WEIGHT		PER CENT OVER + OR UNDER − THEORETICAL WEIGHT
			Ingestion per kilogram	Retention per kilogram	Ingestion per kilogram	Retention per kilogram	
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	
G.O.	5/20/33	16.78	0.70	0.107	0.65	0.099	— 7.6
R.H.	5/20/33	19.01	0.61	0.079	0.64	0.084	+ 6.2
L.T.	5/20/33	15.79	0.68	0.143	0.61	0.129	—11.3
F.W.	7/17/32	14.43	0.54	0.114	0.55	0.116	+ 0.9
D.P.	10/23/32	15.4	0.52	0.060	0.54	0.063	+ 2.9
R.H.	7/17/32	17.4	0.50	0.102	0.54	0.110	+ 8.2
G.O.	3/18/32	16.25	0.49	0.087	0.48	0.086	— 3.3
B.V.	10/ 3/32	18.3	0.47	0.061	0.50	0.065	+ 6.5
J.C.	11/12/32	19.0	0.47	0.062	0.50	0.066	+ 6.4
C.P.	1/16/33	18.0	0.45	0.084	0.46	0.086	+ 2.3
D.B.	10/12/32	17.0	0.44	0.067	0.45	0.070	+ 3.7
D.P.	10/ 3/32	15.1	0.44	0.062	0.45	0.064	+ 1.5
R.H.	4/29/33	18.51	0.45	0.088	0.48	0.094	+ 5.8

weight) retained 0.094 gm. per kilogram. During double metabolism periods (table 2), similar results were obtained; that is, high retentions were not in all cases correlated with underweight.

Objection may be made to the effect that the variation in the nitrogen retentions may be due in part to the kind of protein fed, possibly a larger proportion of high grade protein being given in some cases than in others. The data, therefore, have been considered in relation to the per cent of animal protein (from 49 per cent to 82 per cent) in the various diets. Since it is not feasible to report the data of all the balance studies individually, a few typical records are given (table 4). From these it will be seen that, although the amount of animal protein of the diets differed, this apparently was not the influencing factor in the retention variations. P.D. and P.H.

TABLE 4

Nitrogen retentions of children receiving protein from different sources

NAME	DATE	WEIGHT	PER CENT OVER + OR UNDER − THEORETICAL WEIGHT	NITROGEN INTAKE PER KILOGRAM	PROTEIN ANIMAL	NITROGEN EXCRETION		NITROGEN RETENTION PER KILOGRAM
						Urine	Feces	
		kg.		*gm.*	*per cent*	*gm.*	*gm.*	*gm.*
R.H.	5/12/33	18.8	+ 5	0.607	77	7.465	2.016	0.102
L.T.	5/12/33	16.65	—11.3	0.660	52	7.164	1.534	0.111
C.P.	6/11/33	17.5	— 4.8	0.597	62	7.527	1.192	0.098
R.H.	5/20/33	19.01	+ 6.2	0.606	77	8.357	1.662	0.083
L.T.	3/18/32	14	— 5.4	0.58	82	6.260	0.749	0.081
D.P.	10/23/32	15.4	+ 2.9	0.52	81	5.816	1.264	0.060
B.Mc.	7/17/32	19.12	+ 5.9	0.52	72	6.795	1.451	0.092
G.O.	4/29/33	16.74	— 7.1	0.51	49	5.570	1.507	0.091
B.V.	10/23/32	18.7	+ 6.9	0.49	75	6.588	1.319	0.070
A.C.	2/11/33	18.3	+11.6	0.494	71	5.529	1.335	0.117
E.E.	2/ 3/33	22.6	+ 7.6	0.43	65	7.071	1.204	0.067
J.C.	11/ 4/32	18	+ 0.8	0.43	50	5.118	1.257	0.076
C.P.	5/ 1/32	17	+ 5.3	0.42	62	5.092	1.029	0.061
D.S.	11/ 4/32	17.1	± 0	0.425	53	5.082	1.379	0.047
P.H.	2/20/34	18.74	+ 8.5	0.576	77	8.021	1.251	0.079
P.D.	3/13/34	15.8	— 0.6	0.415	58	4.323	1.033	0.077

retained very similar amounts of protein, 0.077 and 0.079 gm. per kilogram with ingestions of 0.415 and 0.576 gm. per kilogram, respectively. The lower protein diet contained 58 per cent of animal protein while the higher contained 77 per cent.

The relation of nitrogen retention to creatinine elimination

The wide variations in the nitrogen retentions of the children studied even at the same ingestion levels suggest that studies of nitrogen needs based on weight alone may not determine the amount a given child should receive. A thin child may be very well developed and have little padding, while a well-padded child may have less than the normal amount of muscle development. The nitrogen retentions, therefore, have been studied in relation to muscle needs, estimating both ingestion and retention on the basis of the urinary creatinine.

The creatinine determinations by the Folin method were made in duplicate on each of the 24-hour urinary excretions which were collected under toluene and kept on ice, the average of the 5- or 10-day determinations being used. During certain periods, the diets included small amounts of meat given once a day. Tests with meat-free diets indicated that the errors introduced thereby when spread over the 24-hour period were not sufficient to invalidate the results. The nitrogen retention data per milligram of creatinine are grouped for the first and second metabolism periods on the basis of the ingestion per milligram at intervals of five with the corresponding nitrogen ingestions per kilogram of theoretical body weight of the children studied. Double metabolism periods are grouped with the second metabolism periods.

Nitrogen retentions studied from the standpoint of creatinine elimination indicate that at the higher level of ingestion more nitrogen was retained. Retentions during first metabolism periods also were higher than during second subsequent periods, suggesting that some of the children studied had been receiving previously less than the optimum amount of protein.

For example, at ingestion levels between 25 and 29 mg. of nitrogen per milligram of urinary creatinine, 5.1 mg. of nitrogen were retained during first periods and 4.6 mg. during second periods (table 5). At ingestion levels of 35 to 39 mg. of nitrogen per milligram of creatinine, 6.5 mg. and 5.9 mg. were retained during first and second metabolism periods, respectively. At the higher ingestions, similar results were obtained. The significance of the very high retentions at ingestions of 45 to 49 mg. is not clear. The data pertain to three studies with two children, who were above average weight, but somewhat younger than the majority of the children studied. It seems probable that a child of 3 may need more protein per unit of muscle mass than a child of 6. The data based on weights, however, suggest that the variation in age of the children studied was not a significant factor influencing the retentions (table 1).

TABLE 5

Relation between nitrogen ingestion and nitrogen retention based on creatinine excretion

NITROGEN INGESTION PER MILLIGRAM CREATININE	NUMBER OF CASES	AVERAGE AGE	NITROGEN RETENTION			OVER + OR UNDER − THEORETICAL WEIGHT	NITROGEN INGESTION	
			Average per milligram creatinine	Standard deviation	Range per milligram creatinine		Average per kilogram theoretical weight	Standard deviation
First metabolism periods								
mg.		*mo.*	*mg.*	*mg.*	*mg.*	*per cent*	*gm.*	*gm.*
45–49	3	37	11.2	—	9.1–13.0	+ 9.7	0.56	—
40–44	2	40	8.6	—	8.1– 9.0	— 2.8	0.54	—
35–39	15	56	6.5	1.6	4.1–10.0	— 1.0	0.56	0.05
30–34	21	57	5.7	1.2	3.9– 7.5	+ 0.5	0.49	0.04
25–29	15	63	5.1	0.9	2.9– 6.4	+ 1.6	0.47	0.05
20–24	3	63	3.8	—	3.3– 4.6	+ 2.9	0.43	—
Second metabolism periods								
45–49	2	38	9.2	—	8.7– 9.7	+ 1.5	0.55	—
40–44	1	54	6.3	—	———	— 4.9	0.61	—
35–39	18	53	5.9	1.4	3.8– 8.6	— 0.1	0.57	0.04
30–34	20	58	5.0	1.0	3.5– 8.0	+ 0.0	0.51	0.05
25–29	16	62	4.6	0.8	2.8– 6.0	+ 1.2	0.46	0.05
20–24	4	65	3.8	—	2.8– 4.6	+ 7.0	0.44	—

The range of nitrogen retentions based on the creatinine eliminations (table 5; scatter diagram 3) during the second metabolism periods would seem to indicate that ingestions between 30 and 34 mg. per milligram will meet the needs of the average child, since the highest retention of the group,

RETENTION PER KILOGRAM GRAMS	GRAMS 0.35-0.39	0.40-0.44	0.45-0.49	0.50-0.54	0.55-0.59	0.60-0.64	0.65-0.69	0.70-0.74
0.150-0.159					I			
0.140-0.149				I		I	O	
0.130-0.159					I I			
0.120-0.129				I	I O	O		
0.110-0.119			I I O O	I I I I I O O O	I I	△	I I I	
0.100-0.109			I I I	I I I O O	I	I I I		O
0.090-0.099		△	I	I I I O O △	I O △ △	△ △	△	
0.080-0.089		O O	I I O O O O	I O	O	O		
0.070-0.079		I I I I I △ △ △	I O O	O △	O △ △ △			
0.060-0.069	I	I I I I I △ △ O O	I I I O O O O	I I O O	O O △			
0.050-0.059	△	O △ △	I I I O	I	△			
0.040-0.049		I	O					

INGESTION PER KILOGRAM

I FIRST METABOLISM PERIOD
O SECOND METABOLISM PERIOD
△ DOUBLE METABOLISM PERIOD

SCATTER DIAGRAM NO I

SHOWING THE RELATION OF NITROGEN INGESTION TO NITROGEN RETENTION BASED ON THE ACTUAL WEIGHTS OF THE CHILDREN STUDIED

RETENTION PER KILOGRAM GRAMS	GRAMS 0.35-0.39	0.40-0.44	0.45-0.49	0.50-0.54	0.55-0.59	0.60-0.64	0.65-0.69	
0.150-0.159					I			
0.140-0.149					I			
0.130-0.139					I I I O	I		
0.120-0.129				I I O O	I	O O		
0.110-0.119			I O	I I I O	I O O	I		
0.100-0.109			I I	I I I I I I	I I O △	I I		
0.090-0.099		I △	I I O O	I I I O △	I I O △	△	O △	
0.080-0.089			O O O O	O △	O △	△	O	
0.070-0.079		I I △ △ △	I I I I I I O	O O	O △ △			
0.060-0.069	I	I I I	I I I I O O O O O O △	I O O O O △	I I			
0.050-0.059	△ △	O △ △	I I I O	I O	△ △			
0.040-0.049		I	O					

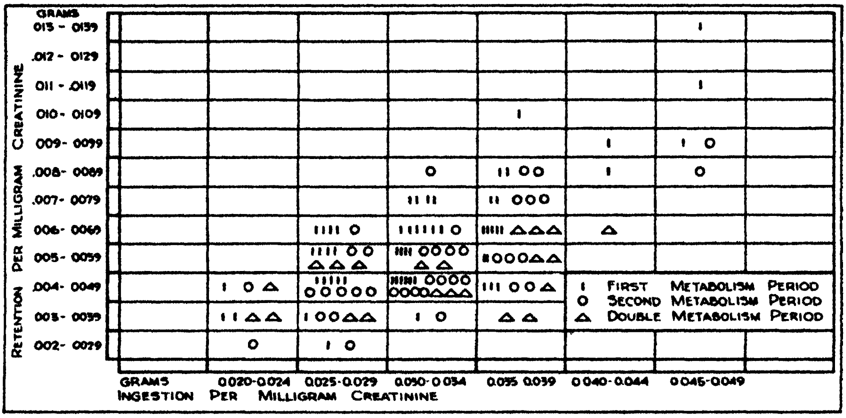
INGESTION PER KILOGRAM

I FIRST METABOLISM PERIOD
O SECOND METABOLISM PERIOD
△ DOUBLE METABOLISM PERIOD

SCATTER DIAGRAM NO II

SHOWING THE RELATION OF NITROGEN INGESTION TO NITROGEN RETENTION BASED ON THE THEORETICAL WEIGHTS OF THE CHILDREN STUDIED

8 mg. per milligram of creatinine is approximately the same as that of the highest retention—8.6 mg. per milligram—of the next higher ingestion level, 35 to 39 mg. Nitrogen ingestions of 30 to 34 mg. per milligram of creatinine considered in relation to the theoretical weights of the children studied were found to be equivalent to 0.51 gm. of nitrogen per kilogram, or 3.2 gm. of protein per kilogram—an amount not far from that reported by Holt in the study of the protein taken by children of similar ages, nor from the ingestions



Scatter Diagram No. III

Showing the Relation of Nitrogen Ingestion to Nitrogen Retention Based on the Urinary Creatinine Elimination of the Children Studied

(0.24 gm. of nitrogen per pound) with which Wang, Hawks and Kaucher ('28) obtained their highest retentions in children of normal weight.

Nitrogen retentions studied from the standpoint of either body weight or muscle development seem to point to the conclusion that children of the preschool age need more protein than we have hitherto appreciated. Instead of 1 gm. per pound, the amount usually considered adequate, approximately 1.5 gm. per pound would seem to be more nearly the requirement of the majority of children of preschool age. The lower levels used in the study as well as those reported by Parsons and Bartlett were sufficient to cover katabolic processes since no child in any of the groups was found to be in negative balance. At these lower levels, furthermore, the children were able to gain in weight and increase in stature. Whether children receiving these lower amounts are less physically fit than those who receive higher levels of ingestion, resulting in higher retentions, remains to be determined.

SUMMARY

In an endeavor to determine the amount of protein which children of preschool age should be given, nitrogen retentions at various levels of ingestion have been studied in children from 37 to 66 months of age. The data have been evaluated, first, in relation to weight, both actual and theoretical; and, second, in relation to the creatinine eliminations which were taken as a measure of muscle needs. The results based on the creatinine eliminations were found to be more consistent than those by other methods tested. Interpreting the data based on creatinine elimination in terms of theoretical weight of the children studied, it is concluded that children of the ages considered should receive approximately 3.2 gm. of protein per kilogram (N.× 6.25) or 1.5 gm. of protein per pound in diets furnishing at least 50 per cent of the protein from animal sources.

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A STUDY OF THE BASAL METABOLISM AND DIET OF NORMAL YOUNG COLLEGE WOMEN IN FLORIDA

JENNIE TILT AND CATHERINE F. WALTERS

Department of Nutrition, School of Home Economics, Florida State College for Women, Tallahassee

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Observations made by Hafkesbring and Borgstrom ('26), Tilt ('30), Remington and Culp ('31), Coons ('31) and Coons and Schiefelbusch ('32) show that the basal metabolic rates of women living in various places in the southern parts of the United States are somewhat lower than the rates of those living in northern parts. The suggestions have been made that a low protein and calorie intake as well as climate might be factors influencing basal metabolism. This study has been made on a group of thirty normal young women, 17 to 26 years of age, for the purpose of adding to the existing data on basal metabolism observations of young women in warm climates. In addition, the average daily protein and calorie intake of eighteen of the group was determined in order to study a possible relationship between basal metabolism and protein and calorie intake.

EXPERIMENTAL

The subjects selected for this study were young women students at the Florida State College for Women, ranging from 17 to 26 years of age. Seventeen of the young women were native Floridians, while thirteen had lived in Florida from 8 to 23 years. Eight of the subjects had recently come from northern states. All subjects were considered normal individuals as shown by health records and examinations by the college physician. Basal metabolism observations were made on the northern group and on thirteen of the Florida group once during the year. Observations were made on the remaining eighteen during the fall, winter, and spring. A 4-day dietary study was made on the group of eighteen in order to determine whether a relationship might exist between diet and basal metabolism.

Basal metabolism. The subjects came to the laboratory early in the morning, 12 to 14 hours after the last meal. After a 40- to 60-minute rest period on comfortable beds, the basal metabolism was observed. A Benedict-Roth apparatus with kymograph attachment was used. Two 8- to 10-minute observations were made during one test period. Four or more observations were made on each subject. The average of those observations agreeing within 5 per cent was considered the basal metabolic rate. Observations during the menstrual period were not avoided unless discomfort was experienced.

Every effort was made to secure accurate determinations. The apparatus was checked for leaks by placing two 20-gm. weights on the spirometer bell for 2 or more minutes during each test. After each morning's observations the residual gas in the spirometer was passed through barium hydroxide or lime water to check for complete carbon dioxide absorption. Since it was impossible to make an alcohol check on the apparatus, two reliable subjects were observed with a Sanborn graphic apparatus in the college infirmary and a Benedict-Roth apparatus at a doctor's office in Tallahassee. The very close checks obtained would indicate that the apparatus was accurate.

The calories per hour were determined and deviations calculated from the following prediction standards: Aub-DuBois, as modified by Boothby and Sandiford ('29); Harris-Benedict, which was extrapolated for those under 21 years of age; and the standard derived from Benedict's Girl Scout studies, making a 10 per cent correction for subjects awake, for those 17 and 18 years old.[1]

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[1] Personal communication.

Dietary study. A study of the protein and calorie intake was made over a period of 4 days, during which time the young women followed their regular dietary habits. All meals were eaten in the college dining hall. The food served at each meal and that eaten between meals was collected and prepared for analysis by the method of Benedict and Farr ('29). The protein constant was determined by the Kjeldahl method (Bodansky, '28); the calorie value by the oxy-calorimeter method (Benedict and Fox, '25). From these data the protein and calorie intake of each subject was calculated.

RESULTS AND DISCUSSION

Basal metabolism. The calories per hour observed on eighteen subjects for different seasons of the year are given in table 1. There appears to be no consistent variation.

TABLE 1

Variation in heat production during fall, winter, and spring

SUBJECT NO.	CALORIES PER HOUR, OBSERVED			
	October–November	February	April	Average
2	50.59	53.52	51.56	51.89
6	46.47	46.70	47.90	47.02
7	50.83	54.23	52.92	52.66
9	44.15	42.45	43.56	43.39
11	52.07	50.34	52.95	51.79
12	50.63	49.72	48.72	49.69
13	49.37	53.61	51.13	51.37
15	50.66	49.25	51.47	50.46
17	54.07	54.10	53.49	53.89
19	47.69	46.31	46.49	46.83
20	48.11	46.57	46.95	47.21
21	48.89	47.99	48.52	48.47
22	48.33	50.22	57.72	52.09
23	57.37	59.61	59.38	58.79
25	44.43	44.93	44.45	44.60
28	53.05	53.76	54.15	53.65
29	51.13	51.06	49.80	50.66
30	48.60	49.79	48.49	48.96

Subjects nos. 2, 7, and 13 show an increase of 5.7 per cent, 6.7 per cent, and 8.6 per cent, respectively, in the basal metabolism observed in February over that observed in October or November. One subject, no. 22, shows an increase of 14.9 per cent in the calories per hour observed in April over the observation in February. It would appear, therefore, that in this climate where temperature changes are not great there is little, if any, seasonal variation. This is in accord with former observations in this laboratory (Tilt, '30). The few variations which do occur cannot be accounted for, since the young women seemed to have made no noticeable changes in their habits.

TABLE 2

Basal metabolism of normal young women living in Florida. Averages according to age group

NUMBER IN GROUP	AGE		HEIGHT	WEIGHT	SURFACE AREA	CALORIES PER HOUR OBSERVED	DEVIATION OF OBSERVED FROM PREDICTION		
							Aub-DuBois modified prediction	Harris-Benedict prediction	Benedict girl scout prediction
	yr.	*mo.*	*cm.*	*kg.*	*sq.m.*		*per cent*	*per cent*	*per cent*
1	17	5	161.0	52.8	1.53	54.92	− 4.0	− 3.6	− 4.0
6	18	5	165.3	56.1	1.61	51.29	−14.5	−13.0	−10.1
6	19	4	165.6	58.1	1.63	49.75	−17.6	−16.4	
7	20	6	163.1	55.6	1.59	49.94	−14.3	−14.0	
5	21	4	165.3	57.3	1.61	51.73	−13.4	−12.1	
2	23	6	163.1	60.8	1.64	53.45	−11.7	−10.1	
2	24	2	165.2	55.5	1.59	52.16	−11.4	− 9.2	
1	25	8	161.0	55.0	1.56	48.96	−13.2	−13.4	
Average							−14.1	−13.0	− 9.2

Since there appeared to be no significant seasonal variation, the calories per hour observed for each season were averaged to obtain the average basal metabolism of these eighteen young women. The results of these determinations, averaged, and those on the additional twelve upon whom observations were made during only one season, all grouped according to age, are given in table 2.

The average deviation of the group is –14.1 per cent from the prediction of Aub-DuBois, modified by Boothby and Sandiford, and –13.0 per cent from the Harris-Benedict standard. There is considerable individual variation from these averages. Eighty per cent of the entire group are below the –10 per cent limit of the Aub-DuBois prediction and 76 per cent from the Harris-Benedict. Fifty per cent (Aub-DuBois) and 43 per cent (Harris-Benedict) are below the –15 per cent limit. Only six of the thirty studied were above –10 per cent (Aub-DuBois), the highest being – 2.2 per cent; three were below – 20 per cent, the lowest – 24.4 per cent. According to the Harris-Benedict standard seven were above –10 per cent, the highest was – 0.2 per cent; three were below – 20 per cent, the lowest observed being – 21.6 per cent. The subject who had the highest metabolism had lived in Florida 10 years, but spends the summers in Indiana.

These results are lower than those previously reported for Florida women of the same ages (Tilt, '30), when an average of –10.6 per cent (Aub-DuBois) and – 9.9 per cent (Harris-Benedict) were found. Although the average is slightly below the –13.5 per cent (Aub-DuBois) reported by Coons ('31) for Oklahoma women of the same age groups, the percentages falling below the –10 and –15 per cent limits are somewhat higher. However, Coons observed lower rates, one – 28.6 per cent (Aub-DuBois) and two cases – 27 per cent. Remington and Culp ('31) reported an average of –10.4 per cent (Aub-DuBois) for a group of South Carolina women.

The results of basal metabolism observations on the group of eight normal young women who had recently come from northern states are given in table 3. It is interesting to note that the average percentage deviation from the Harris-Benedict standard (extrapolated) is – 7.8 per cent, a figure very near the –7.1 per cent obtained by Hetler ('32) and the – 7.6 per cent by Stark ('33) for young women of approximately the same age. The range within the group is from –12.3 per cent to – 2.8 per cent (Aub-DuBois, modified), and –13.5 per cent to –1.7 per cent (Harris-Benedict).

Dietary study. From a study of table 4 which summarizes the average daily intake of each individual, it is noted that most of the diets were quite adequate from the standpoint of grams protein and total calories. The average protein intake is 1.23 gm. per kilogram per day. This value is considerably higher than the 0.94 gm. per kilogram observed by Hetler ('32). It is also somewhat higher than the figures reported for medical students at New Orleans (Denis and Borgstrom, '24), Cleveland (Beard, '27), and Chapel Hill (Brooks, '29), which average slightly above 1 gm. per kilogram. Only three subjects (nos. 7, 11, and 20) ate less than the Sherman ('33) standard of 1 gm. per kilogram of body weight. The quality of the protein consumed appears to be quite adequate for meat, milk, and eggs were well distributed throughout the diets. The calorie intake was calculated on the average daily intake per kilogram of body weight. The average is 39.9 calories. This would be considered sufficient for the activity of these young women. The calorie intake is low for the same subjects whose protein intake is low.

TABLE 3

Basal metabolism of normal young women from northern states. Average according to age groups

NUMBER IN GROUP	MONTHS IN FLORIDA	AGE		HEIGHT	WEIGHT	SURFACE AREA	CALORIES PER HOUR OBSERVED	DEVIATION OF OBSERVED FROM PREDICTION		
								Aub-DuBois modified prediction	Harris-Benedict prediction	Benedict girl scout prediction
	mo.	*yr.*	*mo.*	*cm.*	*kg.*	*sq m.*		*per cent*	*per cent*	*per cent*
4	4	17	3	158.6	51.8	1.50	51.22	− 8.7	−9.9	−10.6
2	10	18	6	164.1	56.6	1.61	53.64	−10.1	−8.9	− 7.2
1	6	19	5	166.0	52.1	1.57	56.10	− 3.9	−1.7	
1	5	21	4	158.2	54.4	1.53	54.84	− 2.8	−3.2	
Average								− 7.7	−7.8	− 9.5

Basal metabolism and protein and calorie intake. There does not appear to be any definite parallelism between protein and calorie intake and basal metabolism. Subject no. 23, who shows a basal metabolism of – 2.4 per cent (Aub-DuBois) and – 0.2 per cent (Harris-Benedict), which is the highest of the group, had an average protein intake of 1.64 gm. per kilogram of body weight per day. This is considerably more than that of others in the group. The daily calorie intake of this subject was also high, averaging 51.6 calories per kilogram of body weight. In the case of subject no. 11, who had the lowest basal metabolism (Aub-DuBois, – 24.4 per cent; Harris-Benedict, – 21.3 per cent), there was a daily intake of 0.96 gm. protein and 29.8 calories per kilogram of body weight. Although the basal metabolism observations were not coincident with the dietary study, it is believed that the diet for the

TABLE 4

Average protein and calorie intake and the basal metabolism of a group of young women living in Florida

SUBJECT NO.	PROTEIN INTAKE		CALORIE INTAKE		AVERAGE BASAL METABOLIC RATE	
	Average per day	Average per kilogram body weight per day	Average per day	Average per kilogram body weight per day	Aub-DuBois prediction	Harris-Benedict prediction
	gm.	*gm.*			*per cent*	*per cent*
2	69.21	1.10	2268	42.1	– 12.1	– 11.4
6	60.49	1.18	1981	39.2	– 16.9	– 16.7
7	49.97	0.82	1662	27.3	– 13.7	– 12.8
9	57.45	1.27	1886	41.5	– 18.5	– 20.0
11	67.70	0.96	2090	29.8	– 24.4	– 21.3
12	56.23	1.09	1960	37.9	– 13.1	– 12.4
13	72.53	1.25	2241	38.6	– 15.1	– 13.7
15	76.75	1.41	2424	45.7	– 11.9	– 11.9
17	77.30	1.48	2520	42.7	– 13.0	– 10.5
19	65.91	1.21	2356	43.2	– 18.6	– 18.1
20	47.77	0.90	1451	27.3	– 16.5	– 16.7
21	62.46	1.38	2184	39.7	– 18.4	– 16.5
22	69.72	1.08	2239	34.7	– 17.6	– 15.4
23	90.94	1.64	2869	51.6	– 2.4	– 0.2
25	60.79	1.12	2148	39.5	– 20.0	– 21.6
28	77.78	1.41	2409	43.5	– 9.7	– 7.2
29	67.90	1.26	2234	40.7	– 13.0	– 11.2
30	80.17	1.49	2720	53.7	– 13.2	– 13.4
Average	67.23	1.23	2202	39.9	– 14.9	– 13.9

4-day period in October was representative of the customary diet while in college, at least. The fact that all of the young women eat in the college dining hall where a carefully planned diet of approximately the same food value throughout the year is served may account for the differences observed by Coons and Schiefelbusch ('32). Their study showed the calorie intake to be barely adequate, and the protein, although adequate in quantity, appeared to be deficient in quality.

SUMMARY

1. Basal metabolic rates were determined on a group of thirty normal young women who have lived in Florida 8 years or longer. The average deviations calculated from prediction standards were: Aub-DuBois, modified by Boothby and Sandiford, –14.1 per cent; Harris-Benedict, –13.0 per cent.
2. Basal metabolic rates were determined on a group of 8 young women who had recently come from northern states. The average deviations were – 7.7 per cent from the modified Aub-DuBois standard, – 7.8 per cent from the Harris-Benedict (extrapolated).
3. A comparison of the average basal metabolism of young women living in Florida with those from northern states shows a decidedly lower rate for Florida women.
4. A 4-day dietary study on eighteen young women of the group living in Florida showed an average intake of 1.23 gm. protein and 39.9 calories per kilogram of body weight per day. The protein was of good quality.
5. No consistent relationship was noted between basal metabolism and protein or calorie intake.

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THE VITAMIN G CONTENT OF HOME-CANNED TOMATO JUICE

CHARLES F. POE AND ESTHER L. GAMBILL
Departments of Chemistry and Home Economics, University of Colorado, Boulder

ONE FIGURE

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There seems to be some disagreement as to the vitamin G content of tomatoes. Hartley ('30) states that tomatoes contain about 0.16 unit of this vitamin; while, on the other hand, Daggs and Eaton ('32) claim that this vitamin is not present in the juice of the tomato. Goldberger and Wheeler ('27), in working with human beings, found tomatoes to possess well-marked pellagra-preventive action when fed in amounts of approximately 15 gm. per kilo of body weight.

The investigation reported in this paper gives the quantitative determination of the water soluble, growth-promoting factor other than vitamin B in home-canned tomato juice.

Ripe, sound tomatoes were gathered from the fields and immediately made into juice. The tomatoes were washed, and any adhering water was removed. They were then quartered, and all blemishes and soft spots were cut out. The tomatoes were crushed and placed in covered pans and were brought quickly to the boiling point. The juice was extracted by means of a rotary sieve, placed in quart jars, and sterilized in live steam for 30 minutes.

Healthy young albino rats, progeny of the Wistar strain, were weaned at 4 weeks and were given the following diet

(Bourquin and Sherman, '31) which is deficient in vitamin G, but adequate in all other factors necessary for growth.

	Per cent
Purified casein	18
Butter fat, water and curd free	8
Cod liver oil	2
Salt mixture, Osborne and Mendel ('19)	4
Cornstarch	68

Alcoholic extract from 50 gm. of wheat for each 100 gm. of food mixture.

The animals were kept on the above diet until the surplus body store of vitamin G had been depleted as indicated by

TABLE 1

Table showing units of vitamin G in home-canned tomato juice

SAMPLE NO.	AMOUNT FED IN CUBIC CENTIMETERS	GAIN IN WEIGHT		UNITS	AVERAGE UNITS
		Total 8 weeks	Weekly average		
		gm.	*gm.*		
1	6	29	3.63	0.20	0.21
	8	43	5.38	0.22	
2	6	24	3.00	0.17	0.17
	8	33	4.13	0.17	
3	6	21	2.63	0.15	0.18
	8	38	4.75	0.20	
4	6	28	3.50	0.19	0.21
	8	44	5.50	0.23	
5	4	24	3.00	0.25	0.25
	6	34	4.25	0.24	
6	4	24	3.00	0.25	0.27
	6	41	5.13	0.29	
7	4	16	2.00	0.17	0.21
	6	34	4.25	0.24	
8	4	20	2.50	0.21	0.21
	6	29	3.63	0.20	
9	4	18	2.25	0.19	0.20
	6	30	3.75	0.21	
10	4	28	3.50	0.29	0.28
	6	37	4.63	0.26	
11	6	25	3.13	0.17	0.18
	8	35	4.38	0.18	
12	6	22	2.75	0.15	0.15
	8	29	3.63	0.15	
13	6	22	2.75	0.15	0.16
	8	33	4.13	0.17	
				Average	0.206

cessation of growth. The animals were then placed in separate metal cages with raised bottoms of wide-meshed wire screen so as to control coprophagy. Each animal was given a daily measured portion of tomato juice in addition to the basal diet, ad libitum.

The animals were weighed three times each week. The total gain in weight for an 8-week period was calculated for each animal. The weights of the animals which showed an average weekly gain nearest to 3 gm. were used to calculate

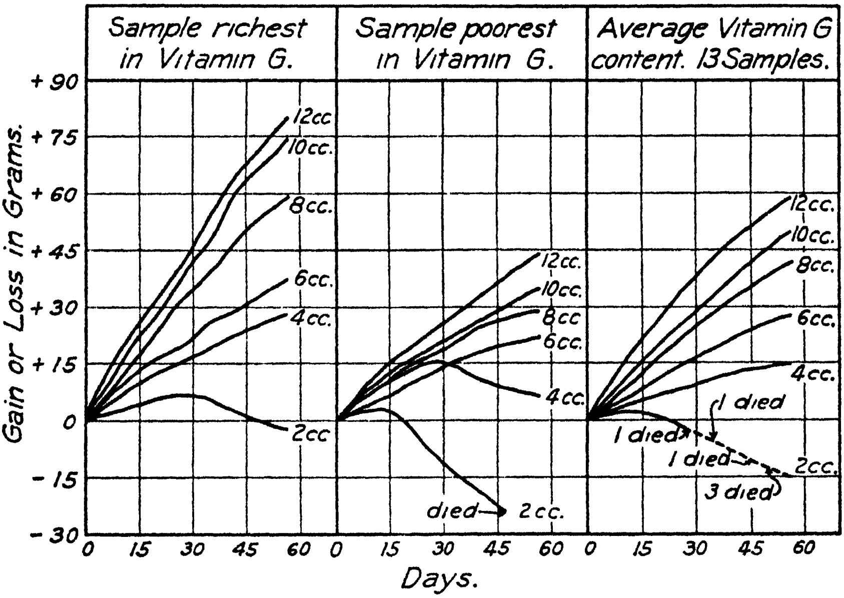

Fig. 1 Growth curves, showing the vitamin G content of tomato juices.

the Sherman units (Bourquin and Sherman, '31) present in each sample. These units are given in table 1.

Figure 1 shows the average growth curves for the animals fed the different samples, and also the growth curves for the animals fed the juices having the maximum and minimum amount of vitamin.

The vitamin content was also determined for the filtered juice, the first four samples being used. No appreciable amount of difference in vitamin content was noted over that found when the whole juice was fed.

It is evident from the data given in table 1 and figure 1 that all of the tomato juices tested contained a fair amount of a water-soluble factor other than vitamin B which promoted growth. Many authors accept this factor as vitamin G. However, as recently set forth in a review by Lewis ('33), vitamins B and G may contain a number of factors. Daggs and Eaton ('32) found a limited amount of a growth-promoting factor present in tomato juice, but stated that it could not be considered as vitamin G because they found definite evidence of dermatitis in feeding amounts of tomato juice up to 7 cc. It appears that these authors really tested their samples for the presence of the old vitamin B-complex, since their basal food was lacking in both vitamins $B(B_1)$ and $G(B_2)$. It seems to the present authors that the growth in the animals studied must have been due to vitamin G, since none of the rats fed 4 cc. or more of the juice developed dermatitis.

A relatively uniform amount of vitamin G was found in all of the samples examined. The minimum amount was 0.15 unit and the maximum amount was 0.28 unit. The average for all samples was 0.21 unit per cubic centimeter or about 95 units per pound.

SUMMARY

1. Thirteen samples of home-canned tomato juice have been assayed for vitamin G.

2. The average vitamin content per cubic centimeter has been found to be 0.21 unit.

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FURTHER STUDIES ON THE EFFECT OF COD LIVER OIL ON THE THYROID GLAND

T. C. SHERWOOD AND W. G. LUCKNER
Department of Anatomy and Physiology, University of Kentucky, Lexington

TWO PLATES (SIX FIGURES)

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It has previously been shown (Moore, '31) that the feeding of an excess of carotene to albino rats results in an accumulation in the liver of large amounts of vitamin A, and that an excess of carotene was found to persist apparently unchanged throughout the alimentary tract. Capper, McKibbin and Prentice ('31) have shown that the fowl as well as the rat can convert carotene into vitamin A. These investigators report that carotene is not stored in the liver unchanged, but is converted into vitamin A. It is obvious, therefore, that conversion to the vitamin takes place subsequent to absorption into the blood stream.

McCarrison ('19) reports that dietaries deficient in vitamins lead to a reduction in size and weight of the thyroid gland.

Kojima ('17) showed that the administration of sodium and potassium iodides caused an accumulation of colloid in the thyroid gland of rats. Chouke ('30) noted that intraperitoneal injection in daily doses of 0.005 gm. and 0.0025 gm. of potassium iodide in rats for periods of 10, 15, 20 and 30 days produced no appreciable change in the structure or in the character of the colloid of the thyroid gland.

Sherwood, Toth and Carr ('34) were able to produce marked changes in the thyroid gland of the rat after administering excessive amounts of cod liver oil.

It was decided, therefore, to investigate, as nearly as possible, the effects of some of the constituents of cod liver oil on the thyroid gland of the rat, and to confirm the results obtained by other investigators (Kojima, '17; Chouke, '30).

EXPERIMENTAL PROCEDURE

The experimental procedure was designed to determine the effect of vitamin A and iodine on the thyroid gland in approximately the same amount as found in cod liver oil. Albino rats received, in addition to our basal ration, excessive amounts of carotene, haliver oil, cod liver oil and potassium iodide.

Since carotene is converted into vitamin A (Moore, '31; Capper et al., '31) caritol (0.3 per cent carotene in oil) was used as a possible source for the vitamin investigation. To further observe the effects of vitamin A, haliver oil (Squibb) was used in one series of animals. Each sample of cod liver oil was analyzed for iodine content and approximately equivalent amounts of potassium iodide were given to another series of adult rats.

Group I. Fifteen animals were killed at regular intervals up to 29 days after having received 0.3 cc. haliver oil daily. This amount of haliver oil contained many times the amount of vitamin A found in the same dose of cod liver oil.

Group II. Seventeen animals were killed at regular intervals from 5 to 45 days after having received 0.1 cc. of caritol daily.

Group III. Six rats were used in order that we might confirm our previous report (Sherwood, Toth and Carr, '34). These animals were killed at 5-day intervals, the experiment covering a period of 30 days.

Group IV. Six rats received approximately the equivalent iodine found in 0.3 cc. of cod liver oil daily. Animals in this series were killed at regular intervals up to 33 days.

Control rats were kept under identical conditions, receiving the same basal diet as in the experimental groups. Control animals of the same age and sex as the experimental animals were killed for thyroid study in each group.

RESULTS

The control animals in each group show the typical normal thyroid gland with an even distribution of colloid and the normal epithelium lining the acini. Figure 1 indicates a normal rat thyroid.

The glands of the animals receiving haliver oil (fig. 2), caritol (fig. 3), and cod liver oil (fig. 4), show somewhat similar pictures, namely, an irregular distribution to a depletion of the colloid.

The cells lining the acini have increased in height and a marked increase in stroma has occurred. Occasionally phagocytic cells are found in the remaining colloid.

The series which received potassium iodide show an increase in colloid after 18 days of experimentation. In 33 days, the acini are strikingly distended, filled with non-vacuolated colloid and composed of extremely low epithelium. This is well demonstrated in figure 5.

DISCUSSION

Vitamin A has been shown to be vital to the growth of animals. It would, therefore, seem possible that some change would be produced in the glandular tissues of the body when diets are lacking in or over-loaded with vitamins. Agduhr ('29) showed that regressive tissue changes were produced with large doses of cod liver oil. The oestrous cycle was apparently affected, since his experimental animals showed no sign of heat.

Carotene and haliver oil have apparently altered the appearance of the thyroid gland, but failed to produce the marked results demonstrated in cod liver oil experiments. This would lead to the conclusion that vitamin A has some effect on the structure of the thyroid. Tissue growth is apparently introduced, since there is an increase of stroma. In the cod liver oil experiment, no increase of stroma is noted, but the epithelium of the acini is increased to more than twice the normal size and a colloid depletion takes place. In a previous report (Sherwood, Toth and Carr, '34) an excess of vitamin D was shown to have no effect on the thyroid gland.

It is of interest to note that the approximate amount of iodine as found in cod liver oil caused an increase in colloid. This is in agreement with the results obtained by Kojima ('17), but contrary to the results obtained by Chouke ('30). In the last mentioned reports far greater amounts of potassium iodide were used.

The iodine of cod liver oil is apparently not as readily available for the metabolic activities as it is in the form of potassium iodide. Much of the iodine of cod liver oil was lost, since the oil was excreted to some extent. This must have been the case, since the feces were very oily.

The minute amounts of iodine had no effect up to the eighteenth day, but following that period all animals showed a decided increase in thyroidal colloid. Apparently, an accumulation finally took place to produce the results obtained.

It has been suggested that the introduction of large amounts of oil might have caused a change in the thyroid structure and that the iodine might have been drawn out into the various fat reservoirs. However, animals receiving large doses of other oils do not show a colloid depletion.

SUMMARY AND CONCLUSIONS

Vitamin A apparently causes some change in the thyroid gland of rats.

Iodine present in the cod liver oil was not responsible for any change in the thyroid gland. However, iodine in the approximate amount found in cod liver oil, given in the form of potassium iodide had a decided effect on the thyroid structure of the rat.

A previous report of the senior author ('34) has been repeated and confirmed.

Due to the various results obtained, it is concluded that carotene and cod liver oil do not produce identical thyroid pictures, but in many respects these are similar.

We have pleasure in expressing our thanks to SMA Corporation, Cleveland, Ohio, for supplying the carotene used in the experiments; to E. R. Squibb & Sons for their generous supply of haliver oil; to Dr. J. S. McHargue for the iodine analyses of the cod liver oil samples; and to Dr. Alfred Brauer for his criticism of the manuscript.

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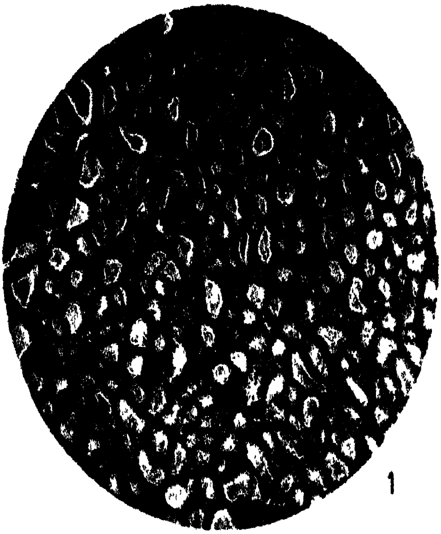
EFFECT OF COD LIVER OIL ON THYROID GLAND
T. C. SHERWOOD AND W. G. LUCKNER

PLATE 1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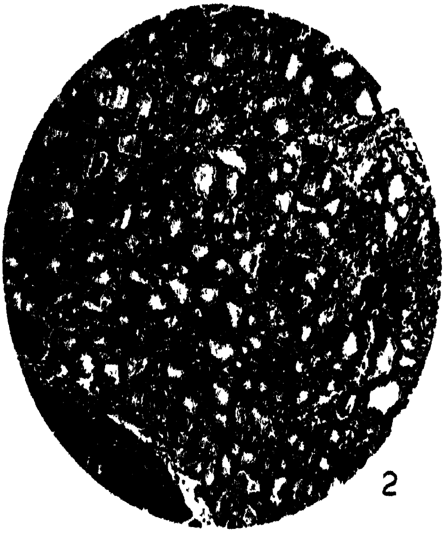
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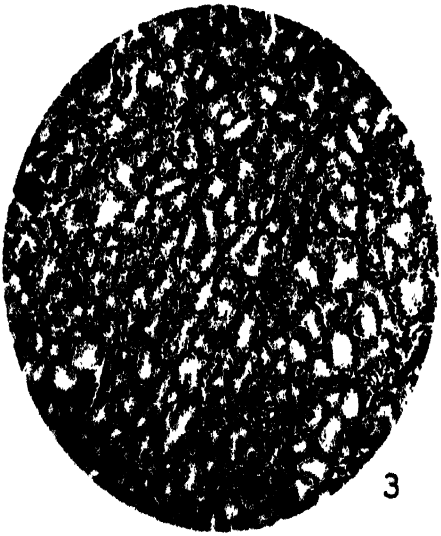
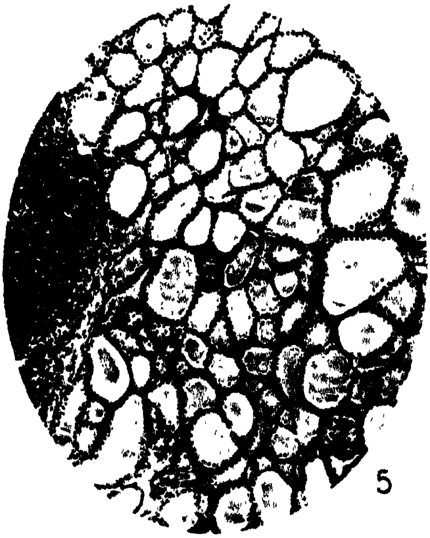
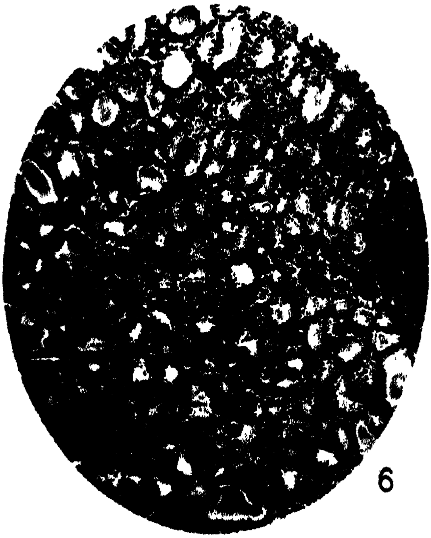

3

1 Photomicrograph of thyroid section, showing normal gland. × 200 approx.

2 Photomicrograph of thyroid section, showing effect of 21-day haliver oil experiment. × 200 approx.

3 Photomicrograph of thyroid section, showing effect of 45-day carotene experimentation. × 200 approx.





4 Photomicrograph of thyroid section, showing effect of 28-day cod liver oil experimentation. × 200 approx.

5 Photomicrograph of thyroid section, showing effect of 33-day potassium iodide experiment. × 200 approx.

6 Photomicrograph of thyroid section, showing effect of 18-day cod liver oil experiment. × 200 approx.

EFFECTS OF LONG-CONTINUED CHOLESTEROL FEEDING IN RATS [1]

WARREN M. SPERRY AND V. A. STOYANOFF
Chemical Laboratory, Babies' Hospital and Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York

TWO FIGURES

(Received for publication July 17, 1934)

Although cholesterol is a normal constituent of all animal food-stuffs, little attention has been paid to the effects on omnivorous animals of long-continued feeding of this sterol in amounts greater than are ordinarily present in the diet. It is well known that in herbivorous animals the ingestion of even small amounts of cholesterol causes a condition similar to arteriosclerosis in humans. It has not proved possible to reproduce this picture in omnivorous animals, but Yuasa ('28) and Schönheimer and Yuasa ('29) have shown that the cholesterol content of the liver increases markedly after long-continued cholesterol feeding in rats, mice and cats. This finding has been confirmed by Okey ('33), Chanutin and Ludewig ('33), and Blatherwick, Medlar, Bradshaw, Post and Sawyer ('33). Okey, Yokela and Knock ('34) have called attention to the possibility that a diet containing large amounts of cholesterol may have a detrimental effect in humans. They found that the cholesterol of the liver is markedly increased in rats after feeding dried egg yolk powder at a high level for about 2 months and conclude, "But egg yolk is being used extremely liberally in diabetic diets at the present time, and we feel that the high incidence of diseases associated with cholesterol deposition in diabetics (e.g., gall stones and

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[1] This investigation was supported in part by the Josiah Macy, Jr., Foundation.

arteriosclerosis) may, at least, not be entirely unrelated to this fact.''

In the present investigation we have studied long-continued cholesterol feeding in relation to growth, efficiency of food utilization, resistance to infection, and deposition of cholesterol in tissues.

I. CHOLESTEROL FEEDING IN RELATION TO GROWTH AND FOOD UTILIZATION[2]

The enormous deposits of cholesterol in the liver, which have been shown in previous and the present (p. 150) investigations to follow cholesterol feeding, might be expected to have an unfavorable influence on growth. The question has been studied in three series of experiments.

Series I was preliminary in nature. Control rats were placed at weaning on a diet composed of extracted casein, sugar, Crisco, salt mixture, Vitavose; and cod liver oil which was fed as a supplement. Casein was prepared according to the technic described by Sperry ('26). Analysis showed that it contained at the most only a trace of cholesterol. An equal number of rats received exactly the same diet, except for the addition of approximately 1.0 per cent of pure cholesterol. The cholesterol used in series I and II was prepared from gall stones by extraction with and several recrystallizations from alcohol. The solution was boiled with charcoal under reflux at the first recrystallization and the last recrystallization was from absolute alcohol. Litters were divided as equally as possible between the two groups and the sex ratio was the same in each.

At the time these experiments were carried out little information was available concerning the distribution of vitamin G and we were under the erroneous impression that Vitavose provided an ample supply of this factor. As was to have been expected, the rats did not grow well; after 162 days twelve rats on the low-cholesterol diet had an average weight of 142 gm., while an equal number on the cholesterol diet averaged 101 gm. The rats receiving cholesterol not only grew less well than the controls, but there was a marked difference in the general appearance of the two groups of animals. The controls, though small, looked perfectly normal. The cholesterol-fed rats, on the other hand, appeared grossly abnormal, as evidenced especially by roughness of coat and inactivity.

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[2] A preliminary report of this part of the investigation was presented before the American Institute of Nutrition at New York, March 28, 1934.

TABLE 1

Composition of diets—series II

SYNTHETIC DIETS			NATURAL-FOOD DIETS		
Constituent	Diet A	Diet B	Constituent	Diet C	Diet D
	gm.	*gm.*		*gm.*	*gm.*
Casein (extracted)	250	250	Oats	150	150
Crisco	200	200	Barley	150	150
Sugar	550	550	Wheat (spring)	150	150
Salt mixture[1]	50	50	Corn (yellow dent)	150	150
Vitavose	100	100	Soy bean meal	150	150
Vitamin Harris	10	10	Alfalfa meal	20	20
Spintrate	4	4	Casein (extracted)	200	200
Viosterol (cc.)	0.2	0.2	Salt mixture[1]	30	30
Cholesterol	0	10	Crisco	100	100
			Cholesterol	0	10

[1] McCollum and Simmonds ('18).

Series II. A group of rats of the Long-Evans strain was paired at weaning according to sex and in most instances according to litter. One-half of the pairs were placed upon synthetic diets A and B (table 1). One member of each pair was given the practically cholesterol-free diet A, while the other received exactly the same diet with cholesterol added (B). The other pairs were fed the natural-food diets C and D.[3] As in the case of the synthetic diets one member of each pair was placed on the cholesterol-free diet C, while the other was given exactly the same diet with cholesterol added (D). In both groups of cholesterol-fed rats analysis of the feces indicated that only a small part of the cholesterol was excreted.

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[3] We are indebted to Dr. T. F. Zucker for suggesting the use of these diets.

The rats were housed in individual cages on wire screens and were fed from 'non-scattering' cups. A sheet of blotting paper was placed on the floor of each cage. The rats and the food consumed were weighed every 2 or 3 days. Any spilled food was collected carefully from the blotting papers.

Series III. In this experiment only male rats of the Wistar strain were used.[4] All rats were paired according to litter and in most instances there was little or no difference in the weights of the two rats in a pair. One rat in each pair was placed at weaning on diet A_1 and the other on diet B_1 which was exactly the same as A_1, except for the addition of cholesterol (table 2). Growth and food consumption were measured as in series II.

TABLE 2

Composition of diets—series III

CONSTITUENT	DIET A_1	DIET B_1
	gm.	*gm.*
Casein (extracted)	200	200
Crisco	250	250
Sugar	365	365
Salt mixture[1]	50	50
Vitavose	100	100
Vitamin Harris	25	25
Spintrate	10	10
Viosterol (cc.)	0.3	0.3
Cholesterol	0	20

[1] Hawk and Oser ('31).

The cholesterol fed in this experiment was purified from a fraction obtained by chilling an alcoholic solution of the unsaponifiable material of cod liver oil, which was furnished through the courtesy of the National Oil Products Company, of Harrison, N. J. It was refluxed in alcohol in the presence of charcoal for many hours and recrystallized several times from alcohol and finally from ethylene chloride. The melting point (corrected) was 148° to 149° and $[\alpha]_D^{20°}$ in ether was — 31.9°. The absence of appreciable amounts of ergosterol was demonstrated by ultraviolet spectrum analysis. During the first 6 weeks of the experiment the cholesterol which was used had also been purified through the bromide. This product had the same physical constants as the unbrominated cholesterol.

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[4] We express our gratitude to Dr. Erwin Brand and to Dr. C. J. Stucky, of the New York State Psychiatric Institute and Hospital, for careful selection of these rats from their colony.

TABLE 3

Effect of dietary cholesterol on growth

AGE	NUMBER OF PAIRS	AVERAGE EXCESS WEIGHT OF RATS RECEIVING NO CHOLESTEROL	PROBABLE ERROR
	Series II—Synthetic diet		
days		*gm.*	*gm.*
20	12	1.2	
40	12	10.3	2.7
60	12	21.4	4.9
80	12	26.8	5.1
100	11	22.4	2.9
	Series III—Synthetic diet		
20	26	— 0.3	
40	24	6.6	2.8
60	18	14.3	4.1
80	18	18.7	4.5
100	14	29.1	5.5
120	8	31.4	9.1
	Series II—Natural-food diet		
20	13	— 4.0	1.6
40	13	— 4.8	2.5
60	13	— 3.1	3.3
80	13	— 4.7	3.7
100	12	— 7.3	3.7

Growth. The growth curves were plotted for each rat, smoothed to rule out day-to-day variations, and the weights at 20, 40, 60, 80, 100 and 120 days were read off. The weights of the cholesterol-fed rats were subtracted from the corresponding weights of their paired controls, the differences were averaged, and the probable errors of the means were computed. The results are tabulated in table 3.

It is evident that rats on the synthetic diets without cholesterol grew better than their cholesterol-fed mates both in series II and III. The average excess weights were significant or nearly so for most of the ages measured after 20 days.

With the natural-food diets cholesterol showed no effect. Indeed, rats receiving cholesterol grew on the average a little better than the controls, but the differences are not significant.

The discrepancy between the findings with synthetic and natural-food diets is most probably related to the fact that the deposits of cholesterol esters found in the livers of rats which had received the natural-food diet with cholesterol averaged only about one-fifth as much as in rats which had received the synthetic diet. In this connection it is of interest that the highest values for cholesterol esters in the liver were found in the cholesterol-fed rats of series I, where the effect of cholesterol feeding on growth was most marked. We have no explanation for the smaller deposits of cholesterol esters on the natural-food diet. The finding that the combined cholesterol of the serum was much lower in cholesterol-fed rats on the natural-food diet than in the corresponding rats on the synthetic diet (table 10) might indicate that cholesterol was less well absorbed in the former, but no marked difference in absorption was indicated by fecal analysis.

Food consumption. The total weight of food (less its cholesterol content) consumed by each cholesterol-fed rat during the first 60 days on the diet was subtracted from the amount consumed by its paired control over the same period. The differences were averaged and the probable errors of the averages were calculated. Differences in food consumption during the first 99 days (series II) and the total period (series III) were determined in the same way. The data (table 4) show that rats on the synthetic diets without cholesterol consumed more food on the average than their controls. The differences were just significant for the 99-day period in series II and for the 60-day period in series III; they were almost significant for the remaining two periods. On the natural-food diet the cholesterol-fed rats ate more on the average than their controls, but the differences were not significant.

These results indicate that animals which grew faster also ate more food. The question arises whether the difference in growth was due entirely to the difference in food consumption or whether some other factor was concerned. Palmer and Kennedy ('31) proposed a formula for estimating the efficiency of food utilization:

$$\text{Efficiency quotient (E. Q.)} = \frac{\dfrac{\text{Digestible dry matter consumed}}{\text{Gain in weight}}}{\text{Mean weight during experiment}} \times 100$$

We felt that the calculation of efficiency quotients according to this formula might answer the above question. Such calculations have been made for the same periods as in the analysis of food consumption. Digestible dry matter was not determined, since the diets compared were the same, except for cholesterol, for which correction was made. Total food consumed was substituted for 'digestible dry matter' in the formula. The difference in E. Q.'s was determined for each pair and the differences were averaged and treated statistically just as in the case of food consumption, except that the E.Q. of the rat which had not received cholesterol was subtracted from the E. Q. of the cholesterol-fed rat. It must be remembered that a higher E. Q. indicates less efficient utilization of food.

TABLE 4

Effect of dietary cholesterol on food consumption

SERIES	DIET	NUMBER OF PAIRS	PERIOD	AVERAGE EXCESS FOOD CONSUMPTION OF RATS RECEIVING NO CHOLESTEROL	PROBABLE ERROR
			days	*gm.*	*gm.*
II	Synthetic	12	60	45.7	14.5
II	Synthetic	11	99	75.5	18.2
III	Synthetic	21	60	31.0	7.8
III	Synthetic	26	Total[1]	35.2	10.1
II	Natural-food	13	60	— 33.0	13.9
II	Natural-food	12	99	— 59.0	21.9

[1] Varied from 27 to 138 days.

The results (table 5) show that for all periods analyzed on both the synthetic and natural-food diets the average E. Q.'s were higher for cholesterol-fed rats than for their controls. The differences were significant for all periods on the synthetic diets, but they were not nearly significant on the natural-food diet as was to have been expected from the inverse relationship found with this diet with respect ~~to growth~~ and food consumption.

The inclusion of the term 'mean weight during experiment' in the calculation of E.Q. had the effect of placing undue emphasis on the weight factor. In our experiments the gain in weight was approximately the same as the mean weight in many instances; consequently the weight factor entered the equation approximately squared. In other words, since in most of our experiments both rats in a pair weighed nearly the same at the start, the average weight was directly related to the gain in weight. Hence we have calculated the average food consumption per gram gain in weight (the upper term in Palmer and Kennedy's formula) for each of the rats and treated the data just as in the case of the E. Q.'s (table 5). The results agree with the E. Q.'s in indicating a decreased efficiency of food utilization in rats fed a cholesterol-containing synthetic diet.

TABLE 5

Effect of dietary cholesterol on the efficiency of food utilization

SERIES	DIET	NUMBER OF PAIRS	PERIOD	AVERAGE DIFFERENCE IN E.Q.'S (E.Q. WITH CHOLESTEROL —E.Q. WITHOUT CHOLESTEROL)	AVERAGE DIFFERENCE IN FOOD CONSUMPTION PER GRAM GAIN
			days		
II	Synthetic	12	60	1.25 ± 0.28	0.53 ± 0.09
II	Synthetic	11	99	0.81 ± 0.16	0.34 ± 0.12
III	Synthetic	21	60	0.31 ± 0.06	0.19 ± 0.04
III	Synthetic	26	Total[1]	0.42 ± 0.07	0.27 ± 0.06
II	Natural-food	13	60	0.31 ± 0.25	0.33 ± 0.13
II	Natural-food	12	99	0.12 ± 0.20	0.37 ± 0.15

[1] Varied from 27 to 138 days.

II. CHOLESTEROL FEEDING IN RELATION TO RESISTANCE TO INFECTION[5]

There is considerable evidence indicating that cholesterol plays an active part in the defense mechanism of the organism. The literature on this subject has been reviewed recently by Degkwitz ('31) and need not be discussed in detail here. In general, it has been found that cholesterol protects red blood cells against hemolytic substances. This action has been well established in the case of saponins (Ransom, '01) with which cholesterol forms non-hemolytic combinations.

Numerous investigators have found that the total cholesterol of the blood is markedly reduced at the height of certain infections. (See Kipp, '20, and Shope, '30, for summaries of the literature; also see McQuarrie and Stoesser, '32.) It has been assumed by many that this decrease is due to a combination of cholesterol with bacterial toxins, similar to the combination with saponins, and Leupold and Bogendörfer ('22), Beumer ('23), Borchardt ('29), and Spranger (quoted by Degkwitz, '31, p. 835) have tested this hypothesis by comparing the resistance of cholesterol-fed and control animals to the same dose of bacterial suspension or toxin. They all found, in confirmation of the theory, that cholesterol-fed animals had greater resistance than controls, as shown by longer periods of survival,[6] and all concluded that cholesterol exerts a general protective action against bacterial infection. Borchardt gives no protocols; the other investigators used a total of thirty-four cholesterol-fed animals and twenty-eight controls. The diets are not described in detail by any of the authors. If the diets received by experimental and control animals differed in other respects than their cholesterol content, the effect may have been due to some other factor which was present in different amounts in the two diets.

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[5] A preliminary report of this part of the investigation was presented before the Society for Pediatric Research at Atlantic City, May 1, 1934.

[6] Levinson ('31) found no effect of cholesterol feeding on resistance to tuberculosis in rabbits. Experiments in which cholesterol sols were injected, such as those of Lubinski and Stern ('26), are open to the criticism that the cholesterol in such emulsions is not in a physiological state. We have limited our discussion and experimental study to the effect of cholesterol feeding.

In view of the practical importance of the conclusion that cholesterol feeding has a general protective action against infection, the relatively small number of experiments on which the conclusion has been based, and the incomplete data concerning the diets, it seemed worth while to investigate the question further. This has been done by inoculating the rats described in part I with the paratyphoid organism Salmonella dansyz,[7] and comparing the survival of control and cholesterol-fed animals. The experiments were designed so that cholesterol might exert its maximum effect; control rats had received a diet containing at the most traces of cholesterol, while experimental rats had received exactly the same diet, except for the addition of cholesterol, for long periods of time (average period 141 days). Under these conditions, the cholesterol-fed rats might be expected to be saturated with cholesterol; that this was the case is shown by the analysis of tissues and blood (part III).

In all of the experiments both control and cholesterol-fed rats in each pair received the same amount of the same culture intraperitoneally. In order that the amount given might be lethal, but not large enough to overwhelm the animals, an attempt was made to determine the minimal lethal dose of the organism. This proved impossible, because the stock rats, which were used for the test, differed markedly in resistance from the rats which had been fed the experimental diets.[8]

We were forced to determine the proper dose by experience with the experimental animals themselves. This was done in series I and II by injecting a pair at a time and adjusting the dose given the next pair according to the results obtained with the first, etc. In most instances the periods of survival were satisfactory. In series III we extended this technic by adjusting the dose on the basis of bacterial counts of the cultures used.[9]

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[7] We are indebted to Dr. Carl ten Broeck, of the Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, for suggesting the use of this paratyphoid organism, because of its general toxicity to rats.

[8] Dr. C. A. Slanetz furnished much valuable advice and assistance in making these tests and in preparing and injecting the cultures.

[9] Miss Florence Fitzpatrick kindly made these counts.

In series I the experiments were complicated by a probable vitamin G deficiency (p. 132). Despite a marked disadvantage in weight and condition, the cholesterol-fed rats showed considerably more resistance than their controls. Three cholesterol-fed rats recovered from the infection, while their much heavier controls, receiving the same amount of culture, died. The average survival of four other cholesterol-fed rats was 128 hours, while the average survival of four controls was 90 hours. There were not enough experiments to justify any conclusions, but the results were so striking in view of the poor condition of the cholesterol-fed rats that it is difficult to believe that they were due entirely to chance. At least they were a stimulus to further studies.

In series II half of the rats had been fed synthetic diets A and B; the other half had received natural-food diets C and D (table 1). In three pairs on the synthetic diets the cholesterol-fed rat survived its control, in seven pairs the contrary was the case, while in one pair both rats survived the same time. In six pairs on the natural-food diets both rats recovered from the infection, in two pairs the cholesterol-fed rat survived its control, and in three pairs the reverse was true. Obviously, cholesterol showed no protective action in this series.

In all cases a pair of rats on the synthetic diet and a pair on the natural-food diet were injected at the same time with the same amounts of the same culture. In seven of the eleven groups all four rats were litter mates. In six of these groups the rats fed natural foods recovered from the infection, while most of the rats fed synthetic diets succumbed. In the remaining five groups, in all of which the rats were somewhat older, no difference in the resistance of rats on synthetic and natural-food diets was evident.

In series III the rats were started at varying times over a period of several months so that animals of different ages might be injected at the same time with the same culture. A few pairs were utilized in determining the proper dose, which was calculated from bacterial counts of the cultures used in

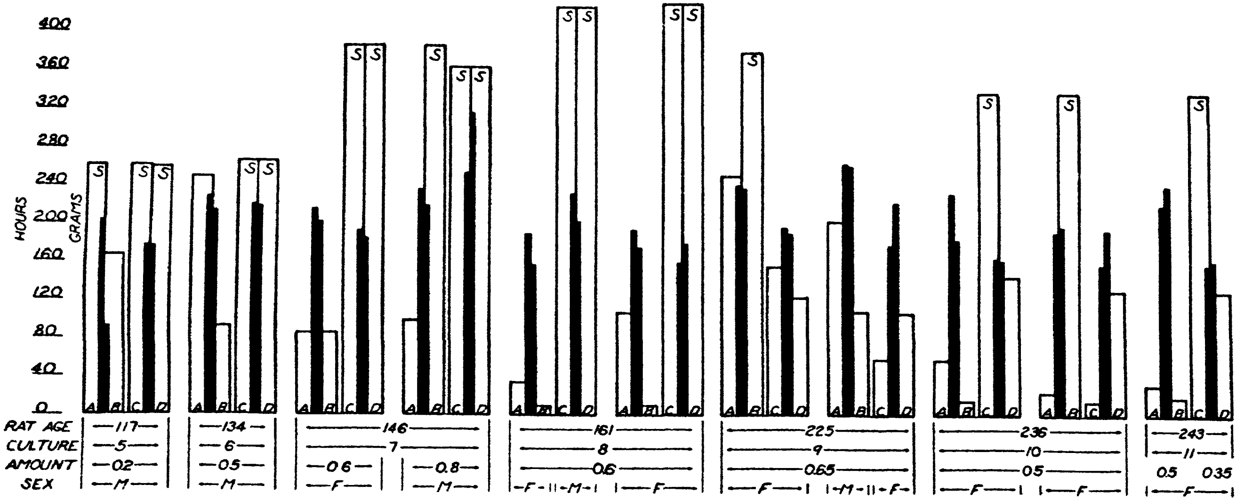

Fig. 1 Survival of rats of series II after inoculation with Salmonella dansyz. Dark bars indicate weight when inoculated. Open bars indicate period of survival in hours. 'S' indicates that rat recovered and was sacrificed. 'A' rats received a synthetic diet without cholesterol. 'B' rats received the same synthetic diet with cholesterol. 'C' rats received a natural-food diet without cholesterol. 'D' rats received the same natural-food diet with cholesterol.

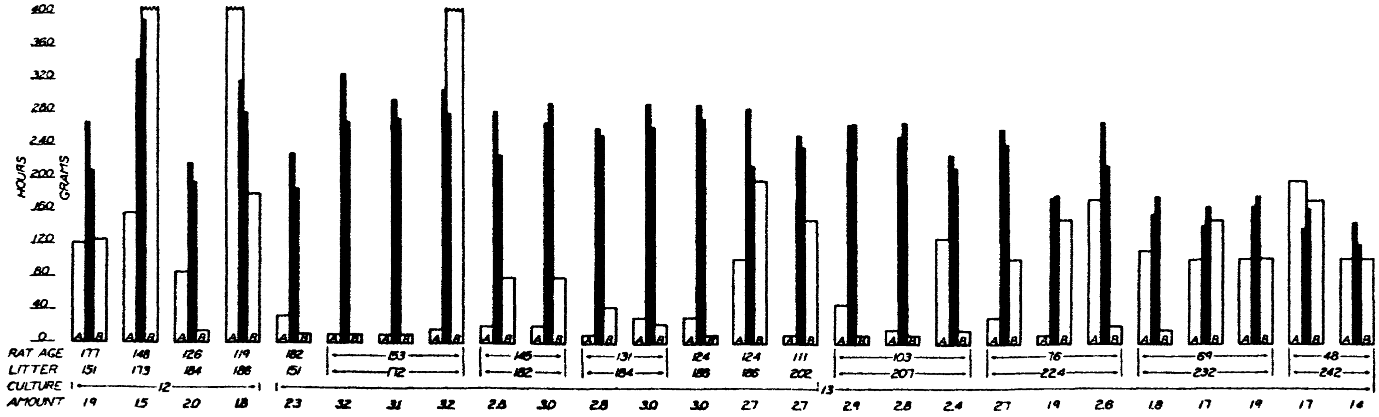

Fig. 2 Survival of rats of series III after inoculation with Salmonella dansyz. Symbols have the same significance as in figure 1.

the preliminary tests and in the inoculation of the main group of rats. The dose was made proportional to the average weight of the two rats of a pair but, as in all of these experiments, both members of a pair received the same amount of culture.

In four pairs there was no difference in time of survival, in eleven pairs the cholesterol-fed rat survived the control, while in eleven pairs the control survived the cholesterol-fed rat. Evidently, as in series II, cholesterol exerted no influence whatsoever. There seemed to be a tendency for the younger rats to show more resistance than the older, but the differences were too small to justify further comment.

Altogether forty-eight pairs of rats were studied in series II and III; in sixteen the cholesterol-fed rat survived its control, in twenty-one the contrary was the case, and in eleven both survived for the same time. Evidently, these results do not support the conclusion under investigation (that cholesterol feeding increases general resistance to infection). We realize that, in view of the large normal variation in the resistance of rats, it is dangerous to draw conclusions from small series of experiments, but we feel that if cholesterol had a general effect in increasing resistance to infection markedly, some evidence of this effect should have appeared in our carefully controlled experiments. It hardly seems likely that in the thirty-two pairs (two-thirds of the total) where the control rat survived as long as or longer than the cholesterol-fed animal, the control rat chanced to have enough excess natural resistance to counterbalance an effect of cholesterol. It is, of course, possible that cholesterol exercises a protective action against other organisms and it may increase resistance to Salmonella dansyz under abnormal conditions, as is suggested by the few experiments of series I, but even if this should be proved beyond doubt, the ingestion of this sterol in excessive amounts as a protective measure by humans is hardly to be recommended in view of the results described in parts I and III of this paper.

III. CHOLESTEROL FEEDING AND INFECTION IN RELATION TO THE CHOLESTEROL OF TISSUES AND BLOOD[10]

Relatively few studies of free and combined cholesterol of tissues have been reported, largely because the only method available until recently (digitonin gravimetric) requires large amounts of tissue and is laborious and time-consuming. The availability of a new, rapid method (Schoenheimer and Sperry, '34) made possible the utilization of the material described in parts I and II in studying the effect of infection and of long-continued cholesterol feeding on tissue cholesterol. Nearly 1000 individual analyses have been made.

Tissues were removed as soon as possible after the death of the animal and a small sample was weighed for moisture determination. In series I and II another sample was weighed, ground with sand, and extracted three times with boiling alcohol followed by three washings with ether. The extracts were filtered into a volumetric flask and made up to volume with alcohol. Free and total cholesterol were determined either by the method of Schoenheimer and Sperry or by a macro-modification thereof. Where the micro procedure was used an aliquot of the alcohol-ether extract was taken to dryness in a small beaker, washed quantitatively with absolute alcohol-acetone (1:1) into a 5-cc. volumetric flask, and made up to volume. From this point on the procedure was the same as described by Schoenheimer and Sperry.

The macro modification was the same in principle as the micro, but the technic differed in several details. It was necessary to concentrate aliquots of the alcohol-ether extract to 2 cc. (free) or 1 cc. (total) in 15-cc. centrifuge tubes. Free cholesterol was precipitated by 1 cc. of 1 per cent digitonin in 40 per cent alcohol. In the determination of total cholesterol alkali was added in the form of sodium wire, the samples were digested 90 minutes at 60°, and acidified with 10 per cent HCl in absolute alcohol. The solution was filtered into another 15-cc. centrifuge tube, using a fritted glass filter with a Pregl siphon, and washing several times with ether, which was removed by drawing a stream of air through the tube. The volume was adjusted to 2 cc. with alcohol and digitonin was added as in the determination of free cholesterol. Centrifuging, washing and color development were carried out in essentially the same manner as in the micro procedure. Five-mm. cells (instead of 5-cm.) were used in reading. (We prefer the micro procedure as described by Schoenheimer and Sperry. We were forced to employ the macro method in the early part of this work as at that time 5-cm. micro cells were not available.)

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[10] A preliminary report of this part of the investigation was presented before the American Society of Biological Chemists at New York, March 29, 1934.

In devising the above procedure a great many control analyses were carried out, similar to those described in connection with the micro method. The accuracy was about the same in both procedures.

Where sufficient material was available most of the determinations were made in duplicate. Duplicates usually agreed within 3 per cent; in a few cases where they differed by more than 5 per cent, the analysis was repeated.

In series III the liver and intestinal tract were removed and the remaining carcass was minced thoroughly by passing several times through a fine grinder. An aliquot was taken for moisture determination and the remainder (or a large aliquot) was extracted completely with hot alcohol in a continuous extractor (Sperry, '26). The alcohol was removed in vacuo, the residue was extracted with ether, the ether solution was dried over sodium sulphate, filtered, and the ether was boiled off. The residue was extracted with petroleum ether, the dried extract was weighed to give total lipids, and an aliquot was dissolved in alcohol-acetone for cholesterol determination as described above. Liver was analyzed by a similar procedure with determination of total petroleum ether-soluble lipids.

Effect of infection on tissue cholesterol. Little is known about the effect of infection on the cholesterol of tissues. Chauffard, Laroche and Grigaut ('12) found low values for the total cholesterol of the adrenals in humans with acute infections. Landau and McNee ('14) confirmed this finding in a few cases and stated that the decrease was largely in the cholesterol esters. A few investigators (Wacker and Hueck, '13), using histological methods, have found a decrease in the cholesterol esters (doubly-refracting bodies) of the adrenal in acute infection in humans. Baumann and Holly ('25) reported that the total cholesterol of the adrenals was definitely reduced in guinea pigs dying of various infections. The same was true of rabbits, though the results were more variable and increases were observed when the animals were sacrificed in the early stages of the infection.

Save for a few isolated determinations in humans (see Bürger, '28, for a summary), other tissues do not appear to have been studied.

In the present investigation analyses of liver, kidney, lung, adrenal, muscle, skin, and the entire carcass (except for liver and intestinal tract) were made. The data (calculated as percentage cholesterol in dry tissue, except in the case of the adrenal) have been grouped arbitrarily according to the period of survival in order to determine whether any significant changes in tissue cholesterol accompanied the infection (table 6). Average values with probable errors are given for the different groups. Analyses of liver, lung, kidney, and adrenal in rats which had received the natural-food diets C and D are not included, because there were too few data in most of the groups to justify comparisons. The results agree in most instances with those presented in regard to influence of infection. A few rats were sacrificed without inoculation as controls. Rats which recovered from the infection may also be considered as controls, since in most cases results from the 'recovered' and control groups agree closely.

In order to conserve space, the data will not be discussed in detail. The infection appeared to cause significant[11] decreases in 1) the combined cholesterol of the liver[12] and 2) the

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[11] The word 'significant' is used to indicate that the difference between averages is more than four times the probable error of the difference.

[12] In the case of cholesterol-fed rats there was also a decrease in the combined cholesterol of the liver, but the differences between groups are not significant, because of a large variation between individuals and a few very high values in the 40- to 120-hour and > 120-hour groups in series I and II. When the largest and best controlled series (III) is considered separately, a highly significant decrease is observed.

TABLE 6

Influence of infection on tissue cholesterol

GROUP	NUMBER OF RATS	DIET[1]	SERIES	CHOLESTEROL		
				Total per cent	Free per cent	Combined per cent
			Liver			
Sacrificed[2]	6	A	I, II and III	0.93 ± 0.15	0.65 ± 0.04	0.28 ± 0.11
Survived < 40 hours	16	A	I, II and III	0.85 ± 0.04	0.70 ± 0.02	0.15 ± 0.02
Survived 40 to 120 hours	13	A	I, II and III	0.83 ± 0.03	0.73 ± 0.03	0.10 ± 0.01
Survived > 120 hours	12	A	I, II and III	0.79 ± 0.02	0.74 ± 0.03	0.05 ± 0.01
Recovered[3]	2	A	I, II and III	0.85	0.73	0.12
Sacrificed	5	B	I, II and III	12.59 ± 2.30	0.89 ± 0.05	11.70 ± 2.27
Survived < 40 hours	16	B	I, II and III	10.89 ± 0.75	1.02 ± 0.03	9.87 ± 0.76
Survived 40 to 120 hours	11	B	I, II and III	8.75 ± 0.99	1.14 ± 0.05	7.61 ± 0.95
Survived > 120 hours	10	B	I, II and III	9.20 ± 2.24	1.34 ± 0.15	7.86 ± 2.11
Recovered	8	B	I, II and III	12.72 ± 1.60	0.84 ± 0.03	11.88 ± 1.35
			Kidney			
Sacrificed	6	A	I and II	1.71 ± 0.07	1.58 ± 0.06	0.13 ± 0.04
Survived < 40 hours	3	A	I and II	1.80	1.78	0.02
Survived 40 to 120 hours	6	A	I and II	1.60 ± 0.05	1.51 ± 0.04	0.09 ± 0.02
Survived > 120 hours	6	A	I and II	1.68 ± 0.07	1.58 ± 0.06	0.10 ± 0.02
Recovered	1	A	I and II	1.81	1.76	0.05
Sacrificed	5	B	I and II	2.18 ± 0.22	1.81 ± 0.18	0.37 ± 0.08
Survived < 40 hours	5	B	I and II	2.20 ± 0.09	1.90 ± 0.03	0.30 ± 0.06
Survived 40 to 120 hours	5	B	I and II	2.19 ± 0.11	1.96 ± 0.14	0.23 ± 0.02
Survived > 120 hours	3	B	I and II	2.02	1.66	0.36
Recovered	6	B	I and II	2.18 ± 0.08	1.85 ± 0.03	0.33 ± 0.06
			Lung			
Sacrificed	6	A	I and II	2.40 ± 0.19	2.17 ± 0.14	0.23 ± 0.03
Survived < 40 hours	3	A	I and II	1.65	1.56	0.09
Survived 40 to 120 hours	6	A	I and II	1.41 ± 0.11	1.23 ± 0.10	0.18 ± 0.02
Survived > 120 hours	7	A	I and II	1.54 ± 0.04	1.38 ± 0.03	0.16 ± 0.01
Recovered	1	A	I and II	2.12	2.00	0.12
Sacrificed	5	B	I and II	2.77 ± 0.12	2.00 ± 0.07	0.77 ± 0.08
Survived < 40 hours	5	B	I and II	2.71 ± 0.11	2.13 ± 0.14	0.58 ± 0.08
Survived 40 to 120 hours	5	B	I and II	2.12 ± 0.11	1.50 ± 0.11	0.62 ± 0.08
Survived > 120 hours	3	B	I and II	3.44	2.23	1.21
Recovered	6	B	I and II	3.52 ± 0.41	2.34 ± 0.13	1.18 ± 0.33
			Adrenal[4]			
Sacrificed	6	A	I and II	5.28 ± 0.62	0.46 ± 0.05	4.82 ± 0.58
Survived < 40 hours	3	A	I and II	0.65	0.26	0.39
Survived 40 to 120 hours	6	A	I and II	0.61 ± 0.08	0.21 ± 0.02	0.40 ± 0.07
Survived > 120 hours	6	A	I and II	0.77 ± 0.05	0.25 ± 0.02	0.52 ± 0.05
Recovered	1	A	I and II	2.95	0.39	2.56
Sacrificed	5	B	I and II	9.39 ± 1.14	0.51 ± 0.07	8.88 ± 1.08
Survived < 40 hours	5	B	I and II	5.16 ± 0.49	0.76 ± 0.09	4.40 ± 0.43
Survived 40 to 120 hours	5	B	I and II	2.03 ± 0.45	0.31 ± 0.04	1.72 ± 0.36
Survived > 120 hours	3	B	I and II	3.32	0.81	2.51
Recovered	6	B	I and II	10.46 ± 1.09	0.65 ± 0.06	9.81 ± 1.06
			Carcass[5]			
Survived < 40 hours	12	A	III	0.41 ± 0.02	0.27 ± 0.01	0.14 ± 0.01
Survived 40 to 120 hours	7	A	III	0.56 ± 0.03	0.35 ± 0.01	0.21 ± 0.02
Survived > 120 hours	5	A	III	0.55 ± 0.09	0.34 ± 0.05	0.21 ± 0.04
Recovered	1	A	III	0.49	0.32	0.17
Survived < 40 hours	9	B	III	0.51 ± 0.02	0.32 ± 0.01	0.19 ± 0.01
Survived 40 to 120 hours	5	B	III	0.66 ± 0.06	0.42 ± 0.04	0.24 ± 0.02
Survived > 120 hours	7	B	III	0.79 ± 0.05	0.50 ± 0.03	0.29 ± 0.03
Recovered	2	B	III	0.37	0.25	0.12

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[1] A was a synthetic, cholesterol-free diet. B was exactly the same, except for the addition of cholesterol.

[2] Rats in the 'sacrificed' groups had not been injected with a bacterial culture.

[3] When sacrificed, rats in the 'recovered' groups were eating well and had recovered completely from the infection as judged by general appearance.

[4] Values for the adrenal are calculated as percentage of fresh tissue.

[5] Liver and intestinal tract were removed. See text.

free cholesterol of the lung in rats which had received no cholesterol in the diet, and 3) in the combined cholesterol of the adrenal both in rats which had and had not been fed cholesterol. Significant increases in both free and combined cholesterol of the carcass accompanied the infection. Muscle and skin were analyzed in rats of series I. There were too few determinations to justify detailed presentation, but it is of interest that the results agree with the findings on the carcass in showing marked increases in cholesterol accompanying the infection. A decrease in the combined cholesterol of the lung and an increase in the free cholesterol of the liver were also noted, but the differences were not significant. There was no change in the kidneys.

TABLE 7

Total lipids in carcass and liver. Series III

GROUP	TOTAL LIPIDS				DIFFERENCE BETWEEN TOTAL LIPIDS AND CHOLESTEROL ESTERS OF LIVER DIET B
	In carcass		In liver		
	Diet A	Diet B	Diet A	Diet B	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Survived < 40 hours	46.6 ± 2.3	50.8 ± 2.3	15.2 ± 0.3	28.9 ± 1.0	13.0 ± 0.3
Survived 40 to 120 hours	35.0 ± 2.1	35.6 ± 2.8	16.8 ± 0.8	23.4 ± 1.1	16.3 ± 0.4
Survived > 120 hours	35.1 ± 3.0	31.0 ± 0.9	14.5 ± 0.6	20.8 ± 1.0	15.3 ± 0.7

We do not feel justified in drawing final conclusions from our data, since there are too few determinations in many of the groups to satisfy strict statistical requirements. A transport of cholesterol from glandular tissue, such as liver and adrenal, probably to the muscles and skin under the influence of the infection is indicated.

Effect of infection on total (petroleum ether-soluble) lipids. Petroleum ether-soluble lipids of the carcass and liver were determined in series III. The results are summarized in table 7. The lipids of the carcass decreased as the infection progressed, but the differences are not statistically significant in the case of rats which had not received cholesterol. It is surprising that the decreases were not larger, in view of the severity of the infection and the failure, for the most part, of the animals to eat.

There was no appreciable change in liver lipids of rats which had received no cholesterol. In rats which had received cholesterol there was a significant decrease as the infection progressed. The individual percentages of cholesterol esters (calculated as cholesterol oleate from the combined cholesterol values) were subtracted from the corresponding total lipid percentages. The average differences are very nearly the same as the total lipid percentages found in rats which had not received cholesterol. The variation is less than was shown by the total lipid values. Apparently, the increase in liver lipids on the cholesterol diet and the decrease accompanying the infection were entirely in the cholesterol ester fraction.

The influence of cholesterol feeding on the cholesterol of tissues. Several investigators have reported recently that long-continued cholesterol feeding causes large increases in the cholesterol of the liver (p. 131). In agreement with this work, our data show an enormous increase in combined cholesterol of the liver in rats which received cholesterol in the diet (table 6).

Chanutin and Ludewig ('33) found the total lipids of the liver to be increased markedly by cholesterol feeding. Calculation from average percentages shows that the increase was considerably greater than can be accounted for by increased cholesterol esters. Okey ('33) also found a greater increase in the total fatty acids of the liver than the fatty acids combined with cholesterol could explain. In our experiments the increase in liver lipids following cholesterol feeding was very nearly the same as the increase in cholesterol esters (table 7). We have no explanation of this discrepancy. The negligible amount of cholesterol esters in the livers of rats which had not received cholesterol was disregarded in making these calculations.

In this connection it is interesting that cholesterol feeding caused no increase in the lipids of the carcass.

Okey reports no effect of cholesterol feeding on the free cholesterol of the liver, while Chanutin and Ludewig found an increase. Our data on free cholesterol of the liver of rats which had not received cholesterol have been compared with the corresponding values for rats which had received cholesterol (table 8). The results have been tabulated by groups according to the survival of the animals in order to rule out the effect of the infection as far as possible. In all groups the percentages are higher in the case of cholesterol-fed rats than in rats which had not received cholesterol and in most instances the differences are significant.

TABLE 8

Effect of cholesterol feeding on free cholesterol of liver

GROUP	SERIES	FREE CHOLESTEROL		DIFFERENCE	PROBABLE ERROR OF DIFFERENCE
		Without cholesterol in diet	With cholesterol in diet		
		per cent	*per cent*		
Sacrificed	All	0.65 ± 0.04	0.89 ± 0.05	0.24	0.06
Survived < 40 hours	All	0.70 ± 0.02	1.02 ± 0.03	0.32	0.07
Survived 40 to 120 hours	All	0.73 ± 0.03	1.14 ± 0.05	0.41	0.06
Survived > 120 hours	All	0.74 ± 0.03	1.34 ± 0.15	0.60	0.15
Survived < 40 hours	III	0.71 ± 0.02	0.96 ± 0.02	0.25	0.03
Survived 40 to 120 hours	III	0.74 ± 0.04	1.02 ± 0.05	0.28	0.06
Survived > 120 hours	III	0.73 ± 0.06	1.03 ± 0.06	0.30	0.08

Chanutin and Ludewig are the only authors who have studied the effect of cholesterol feeding on the cholesterol of other tissues than liver in the rat. They conclude that there were no changes in the tissues which they studied (kidney, heart and brain). We have analyzed our data on other tissues in the same manner employed for free cholesterol of the liver (table 9). Cholesterol-fed rats showed a higher average cholesterol content than controls in every group compared, except one. Although in the majority of cases the differences are not statistically significant, due to a considerable individual variation, we feel that the consistency of the results and the relatively large percentage increases in most cases indicate a tendency for cholesterol to deposit in tissues generally during long-continued cholesterol feeding. These deposits are, however, insignificant when compared with the enormous increases in combined cholesterol of the liver. In several of our cholesterol-fed rats over half of the total body (except intestinal tract) cholesterol was present in the liver.

TABLE 9

Effect of cholesterol feeding on the cholesterol content of various tissues

GROUP	TISSUE	CHOLESTEROL FRACTION	CHOLESTEROL		INCREASE ON CHOLESTEROL DIET
			Without cholesterol in diet	With cholesterol in diet	
			per cent	*per cent*	*per cent*
Sacrificed	Kidney	Total	1.71 ± 0.07	2.18 ± 0.22	27.5
Survived 40 to 120 hours	Kidney	Total	1.60 ± 0.05	2.19 ± 0.11	36.9
Sacrificed	Kidney	Free	1.58 ± 0.06	1.81 ± 0.18	14.6
Survived 40 to 120 hours	Kidney	Free	1.51 ± 0.04	1.96 ± 0.14	29.8
Sacrificed	Kidney	Combined	0.13 ± 0.04	0.37 ± 0.08	184.6
Survived 40 to 120 hours	Kidney	Combined	0.09 ± 0.02	0.23 ± 0.02	155.6
Sacrificed	Lung	Total	2.40 ± 0.19	2.77 ± 0.12	15.4
Survived 40 to 120 hours	Lung	Total	1.41 ± 0.11	2.12 ± 0.11	50.4
Sacrificed	Lung	Free	2.17 ± 0.14	2.00 ± 0.07	—7.8
Survived 40 to 120 hours	Lung	Free	1.23 ± 0.10	1.50 ± 0.11	22.0
Sacrificed	Lung	Combined	0.23 ± 0.03	0.77 ± 0.08	234.8
Survived 40 to 120 hours	Lung	Combined	0.18 ± 0.02	0.62 ± 0.08	244.4
Sacrificed	Adrenal	Total	5.28 ± 0.62	9.39 ± 1.14	77.8
Survived 40 to 120 hours	Adrenal	Total	0.61 ± 0.08	2.03 ± 0.45	232.8
Sacrificed	Adrenal	Free	0.46 ± 0.05	0.51 ± 0.07	10.9
Survived 40 to 120 hours	Adrenal	Free	0.21 ± 0.02	0.31 ± 0.04	47.6
Sacrificed	Adrenal	Combined	4.82 ± 0.58	8.88 ± 1.08	84.2
Survived 40 to 120 hours	Adrenal	Combined	0.40 ± 0.07	1.72 ± 0.36	330.0
Survived < 40 hours	Carcass	Total	0.41 ± 0.02	0.51 ± 0.02	24.4
Survived 40 to 120 hours	Carcass	Total	0.56 ± 0.03	0.66 ± 0.06	17.9
Survived > 120 hours	Carcass	Total	0.55 ± 0.09	0.79 ± 0.05	43.6
Survived < 40 hours	Carcass	Free	0.27 ± 0.01	0.32 ± 0.01	18.5
Survived 40 to 120 hours	Carcass	Free	0.35 ± 0.01	0.42 ± 0.04	20.0
Survived > 120 hours	Carcass	Free	0.34 ± 0.05	0.50 ± 0.03	47.1
Survived < 40 hours	Carcass	Combined	0.14 ± 0.01	0.19 ± 0.01	35.7
Survived 40 to 120 hours	Carcass	Combined	0.21 ± 0.02	0.24 ± 0.02	14.3
Survived > 120 hours	Carcass	Combined	0.21 ± 0.04	0.29 ± 0.03	38.1

The cholesterol of the blood serum was determined in rats which were sacrificed without infection and which recovered from the infection (table 10). Cholesterol feeding caused large increases in all cholesterol fractions. The effect was much more marked with synthetic than with natural-food diets (p. 136).

It is evident that omnivorous animals, such as the rat, do not differ from herbivorous animals in the ability to absorb cholesterol and deposit it in tissues. They appear to differ markedly in regard to cholesterol deposits in the arteries. Aortas from most of the cholesterol-fed rats were examined by Dr. Beryl Paige, of the Department of Pathology, Babies' Hospital. No arteriosclerotic lesions were discovered. Under the same dietary conditions, marked lesions, involving large cholesterol deposits, would have been expected in rabbits.

TABLE 10

Cholesterol in serum

GROUP	NUMBER OF RATS	DIET	CHOLESTEROL		
			Total mg./100 cc.	Free mg./100 cc.	Combined mg./100 cc.
Sacrificed	5	A	59.9 ± 2.6	18.4 ± 1.9	41.5 ± 1.0
Recovered	2	A	59.1	17.3	41.8
Sacrificed	5	B	231.4 ± 29.1	48.1 ± 8.1	183.3 ± 22.3
Recovered	8	B	211.7 ± 14.7	56.1 ± 4.4	155.6 ± 10.9
Sacrificed	2	C	59.6	18.1	41.5
Recovered	8	C	51.2 ± 2.8	12.4 ± 1.0	38.8 ± 2.2
Sacrificed	2	D	95.0	20.8	74.2
Recovered	6	D	108.1 ± 11.0	25.6 ± 2.6	82.5 ± 8.6

SUMMARY

Long-continued ingestion of cholesterol at a high level has been studied in relation to growth, efficiency of food utilization, resistance to infection, and deposition of cholesterol in tissues.

Cholesterol-fed rats grew less well, ate less food, and utilized their food less efficiently than controls receiving the same synthetic diet without cholesterol. These effects are most probably related to the large deposits of cholesterol esters found in the livers of the cholesterol-fed rats, since they were not observed in a group of rats (receiving a natural-food diet plus cholesterol) in which the deposits of cholesterol were relatively small.

Except in a small series complicated by a probable vitamin G deficiency rats fed diets high in cholesterol for long periods of time showed no more resistance to the paratyphoid organism Salmonella dansyz than controls fed exactly the same diet without cholesterol.

Free, total and combined cholesterol have been determined in various tissues of the rats used in the foregoing studies. Decreases in the cholesterol of liver, adrenal and lung and increases in the cholesterol of the entire carcass (except for liver and intestinal tract) accompanied the paratyphoid infection. No changes were observed in kidney cholesterol.

Increases in all cholesterol fractions of all tissues studied followed cholesterol feeding, but the increase was large only in the combined cholesterol of the liver.

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THE EFFECT OF DIET ON LIVER CHOLESTEROL IN CHICKENS[1]

WARREN M. SPERRY AND V. A. STOYANOFF

Chemical Laboratory, Babies' Hospital and Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York

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Little attention has been paid to the cholesterol of bird tissues or the effect of diet thereon. Several investigators (Yamagouchi, '25; Anitschkow, '25; Uchiyama, '30) have reported deposits of cholesterol esters (anisotropic droplets) in the livers of chickens and pigeons following the feeding of cholesterol. From these results it would appear that cholesterol is absorbed and deposited by birds as it is by mammals; but the only chemical analyses, of which we are aware, are those of Hotta ('24), who determined the total cholesterol of a number of tissues in the pigeon following cholesterol feeding. Marked increases in the cholesterol of liver, muscle, spleen, gonads and blood, and small increases in adrenal, pancreas, and brain cholesterol followed the ingestion of large quantities (0.25 to 0.50 gm. per pigeon per day) of cholesterol over a period of about 1 month. Unfortunately, from the standpoint of the problem at hand, these experiments were complicated by the presence of beri-beri. The diet consisted only of polished rice and cholesterol. The experiments were suggested by the finding of Lawaczeck ('23) and Hotta ('23) (later confirmed by Messerle, '25) that the cholesterol of various tissues (liver, muscle, spleen, pancreas, brain and blood) is increased in beri-beri. The increases found by Hotta after cholesterol feeding were little, if any,

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greater in most tissues than the increases caused by beri-beri per se.

We have been fortunate in obtaining the livers[2] from a number of chickens which had been fed diets varying widely in cholesterol content under carefully controlled conditions in the course of an investigation of the nutritional encephalomalacia described by Pappenheimer and Goettsch ('31, '33, '34). Free, total, and combined cholesterol have been determined in these livers.

EXPERIMENTAL

The extraction of the livers and the determination of cholesterol were carried out in the manner described in the preceding paper (Sperry and Stoyanoff, '35). The micro method was used exclusively for the cholesterol analyses.

DISCUSSION

The results, expressed as percentage of dry weight of liver, are presented in table 1. On diet 634, which was low in cholesterol, the values are quite uniform. The total cholesterol percentages are about the same as those found in rats which received a synthetic diet without cholesterol (diet A) (table 6 of the preceding paper), but the free cholesterol is slightly higher and the combined cholesterol is lower.

Diet 108, although low in cholesterol, probably contained more of this sterol than diet 634, since it was comprised largely of substances (lard, casein and skimmed milk powder) containing small percentages of cholesterol. A definite tendency to deposit cholesterol esters in the liver was exhibited by chicks on this diet. Most of the values are higher than were found with diet 634. (Note especially chick 1409.)

The commercial lecithin, which was fed along with diet 108 in the third series, may have contained small amounts of cholesterol. Most of the chicks showed no greater deposits than the controls on diet 108, but both free and combined

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[2] We are greatly indebted to Dr. Alwin M. Pappenheimer and Dr. Marianne Goettsch for this material.

TABLE 1

Cholesterol in chicken livers on different diets

CHICK NO.	DAYS ON DIET	CHOLESTEROL		
		Total per cent	Free per cent	Combined per cent
Natural-food diet 634[1]				
1488	33	0.833	0.776	0.057
1362	37	0.908	0.855	0.053
1475	37	0.928	0.844	0.084
1479	37	1.021	0.942	0.079
1485	44	0.783	0.735	0.048
1477	53	0.718	0.676	0.042
Diet 108[1]				
1408	26	1.071	0.873	0.198
1415	28	0.879	0.786	0.093
1413	49	0.996	0.835	0.161
1404	50	1.011	0.829	0.182
1402	56	1.000	0.942	0.058
1407	56	0.846	0.714	0.132
1409	56	1.471	1.143	0.328
1414	59	0.992	0.802	0.190
1418	59	0.896	0.826	0.070
Diet 108 + 0.1 gm. lecithin[2] per chick per day				
1436	24	1.209	0.996	0.213
1439	25	0.972	0.851	0.121
1448	30	1.648	1.535	0.113
1441	34	1.020	1.044	....
1454	41	0.955	0.835	0.120
1452	42	1.051	0.923	0.128
1456	42	0.858	0.757	0.101
1437	47	2.898	1.818	1.080
1446	49	0.865	0.844	0.021
1453	49	1.702	1.256	0.446
Diet 108 + 1 cc. egg yolk per chick per day				
1432	40	4.177	2.578	1.599
1421	47	0.811	0.708	0.103
1426	49	1.113	0.911	0.202
1420	50	0.931	0.795	0.136
1423	50	1.107	0.847	0.260
1424	50	0.955	0.852	0.103
1428	50	0.909	0.810	0.099
1430	50	0.982	0.804	0.178
1433	50	0.704	0.644	0.060
Diet 108 + 5 gm. calf brain per chick per day				
1466 1473 1460	24	6.891	1.874	5.017
1469	42	1.691	1.086	0.605
1463	44	3.024	1.888	1.136
1457	47	1.560	1.226	0.334
1458	59	1.264	1.000	0.264
1459	59	1.599	1.353	0.246
1461	59	1.613	1.148	0.465
1462	59	4.116	1.660	2.456
1464	59	1.949	1.277	0.672
1465	59	1.784	1.251	0.533
1468	59	1.130	0.891	0.239
1471	59	3.098	1.340	1.758
1472	59	2.817	1.599	1.218
1467	61	2.201	1.664	0.537
1470	61	1.694	1.399	0.295

[1] See Pappenheimer and Goettsch ('33) for the composition of diets 634 and 108.

[2] Commercial lecithin was used.

cholesterol were exceptionally high in the case of chick 1437, while the free cholesterol was elevated in the case of chick 1448.

The 1 cc. of egg yolk fed with diet 108 in the fourth series may be assumed to have contained approximately 15 mg. of cholesterol since most published analyses of egg yolk show a cholesterol content of about 1.5 per cent. This amount showed no effect, except in chick 1432, where very high values for both free and combined cholesterol were obtained.

There is wide variation in the published analyses of cholesterol in brain. The average is about 2 per cent, which would be equivalent to an extra intake of 100 mg. of cholesterol per chick per day in the fifth series where 5 gm. of brain were fed along with diet 108. There was an unmistakable increase in all liver cholesterol fractions in most of the chicks on this diet, but with a few exceptions the deposits of combined cholesterol were relatively small. The increase was as marked in the free as in the combined cholesterol, in contrast to the finding with rats where the increases in free cholesterol after cholesterol feeding were relatively small (table 8 of the preceding paper). The largest deposits were found in the three chicks (1466, 1473 and 1460, pooled for analysis) which had been on the diet only 24 days as compared with 42 days or more in the case of the remaining chicks. The livers of these chicks were loaded with anisotropic bodies. In this connection it is interesting that Schönheimer and Yuasa ('29) found that the blood cholesterol reached a maximum after about 40 days of cholesterol feeding and then fell off rapidly in rabbits. Unfortunately, no liver analyses were done.

SUMMARY

Total, free and combined cholesterol have been determined in the livers of chicks which had been fed diets of varying cholesterol content.

The results show that chickens behave like mammals in absorbing cholesterol and depositing it in the liver, but they differ from rats in depositing proportionately more free cholesterol.

Occasional chicks showed exceptionally high cholesterol values on diets which were low in cholesterol, indicating a marked individual variation in ability to assimilate this sterol.

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VITAMINS A, D AND B IN OYSTERS—EFFECT OF COOKING UPON VITAMINS A AND B[1]

DOROTHY V. WHIPPLE

Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia

THREE FIGURES

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Oysters are one of the few animal foods in which the entire animal is eaten. It has been repeatedly demonstrated (McCollum, '31) that the visceral parts of animals usually contain larger vitamin reserves than the muscle meat. Since no part of an oyster, except the shell, is discarded in preparing it for food, the active tissue as well as the storage tissue is consumed. The food of the oyster consists of marine plankton of which diatoms are an important part (Savage, '25). To these minute forms has been attributed the original synthesis of the fat-soluble vitamins which certain fish, notably the cod and the halibut, are able to concentrate in their livers (Coward and Drummond, '21).

The American oyster, Ostrea virginica, inhabits the tidal waters of bays and estuaries of the United States from Massachusetts to the Gulf of Mexico. With very few exceptions, all the marketable oysters from the Atlantic coast waters are of the same species, but, pursuant to varying local customs within the industry, oysters north of the Chesapeake are maintained on the beds for from 3 to 5 years before marketing, whereas further south the stock is harvested at from 1 to 2 years of age. The English species, O. edulis, and the American Pacific coast oyster, O. lurida, are quite similar. The only other oyster of commercial significance on this continent is the Japanese oyster, O. gigas, of the west coast.

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[1] This investigation was conducted in part under a grant from Dr. H. D. Pease, director of the Research Committee of the Oyster Growers and Dealers Association of North America.

Each species, and, indeed, oysters of the same species from various sections, has its individual market where its particular qualities of size, shape and flavor are in demand.

Jones, Murphy and Nelson ('28) made a survey of the vitamin content of shellfish in 1928 and their work indicated that the southern oysters with which they worked contained good stores of vitamins A, B and D. Randoin ('23) demonstrated vitamin C in oysters from French tidal waters in 1923.

Methods of vitamin assay have been further standardized since Jones, Murphy and Nelson made their studies, and, in addition, vitamin B has been separated into more than one component. It was, therefore, thought of interest to review the problem of the vitamin content of oysters, using O. virginica from northern waters for the study. Since a large percentage of this food is cooked before it is eaten, the effect of this treatment on the heat-sensitive vitamins was included in the present investigation.

METHODS

Oysters.[2] A haul of oysters large enough for all the present studies was taken from Great South Bay, Long Island, New York, in October of 1932, immediately shucked, washed in sea water, and 'quick-frozen' in half-pound packages by the 'Birdseye process' at a temperature of — 33°C. The frozen oysters were kept at a temperature of — 10°C. until used. When biological material is frozen at a temperature as low as — 33°C., it passes through the critical range so rapidly that ice crystals do not form and hence damage to cell structures is almost completely avoided.

For the vitamin assays, graded doses of raw and cooked oysters were fed to rats. A mass of raw, frozen oysters was ground up in a mortar, weighed out, and fed immediately. Cooked oysters were prepared as follows: Six whole oysters were removed from the package, placed in a beaker with no additional water, and kept on a steam bath for 10 minutes at which time the edges of the oysters' mantles curled. They were then removed, immediately ground in a mortar, weighed and fed. All moisture which exuded from the oysters during cooking was thoroughly mixed with the meats during grinding. The rats consumed the oysters very readily.

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[2] We are indebted to Mr. W. H. Raye, president of the Bluepoints Company, for this supply of oysters.

Chemical analyses were made of the frozen oysters.

TABLE 1

Chemical composition of raw oysters

	Per cent
Moisture	77.35
Total solids	22.65
Total nitrogen	1.59
Protein (N × 6.25)	9.95
Fat	1.45
Ash	1.69
Salt (NaCl)	0.69
Glycogen	6.14
Calcium as CaO	0.10
Magnesium as MgO	0.09
Sulfur (S)	0.09
Phosphorus as P_2O_5	0.30
Lead	Absent
Mercury	Absent
Arsenic	1 part per million
Copper	67 parts per million
Iron	33 parts per million
Aluminum	134 parts per million

The moisture content of the oysters after cooking was 75.00 per cent.

Rat assays of vitamin A, B and D

The rats were of Wistar Institute strain. Since growth is used quantitatively as a criterion of the vitamin content for vitamins A and B, the treatment of the rat colony and of the young rats previous to the experiment is important and is therefore given. The breeding colony was kept on the following diet:

Colony diet

	Per cent
Whole ground wheat	35
Whole ground corn	32
Whole milk powder	21
Alfalfa leaf meal	2
Old process linseed oil meal	7
Liver, vacuum dried	0.5
Yeast	2.0
$CaCO_3$	0.5
NaCl	0.5

When litters were 10 days old, the mother was transferred to

Vitamin restricted diet

	Per cent
Whole ground wheat	33
Whole ground corn	34
Skimmed milk	21
Old process linseed oil meal	7
Yeast	4
$CaCO_3$	0.5
NaCl	0.5

The young rats were weaned at 21 days of age, at which time the mother was put back on the Colony diet and the young were continued on the vitamin restricted diet until they were 28 days of age. Only young rats weighing between 40 and 50 gm. were used for the experiment.

When ready for the experiment, the young rats were transferred to the vitamin-deficient ration. Rats to be used for the vitamin A and B experiments were allowed to remain on the deficient ration until growth began to decline. At that time, they were divided into groups of eight to ten rats, comparable with respect to sex and litter, and fed graded doses of raw and cooked oysters. One group was always kept as a negative control, and one group was given a known amount of standard source of the vitamin in question.

Our vitamin-deficient diets were as follows:

Vitamin A deficient diet

	Per cent
Casein (vitamin A and B free)	18
Starch	60
Yeast	8
Salt mixture (Osborne and Mendel)	4
Lard	8
Viosterol (1: 1000)	2

Vitamin B(B_1) deficient diet

	Per cent
Casein (vitamin A and B free)	18
Starch	53
Autoclaved yeast	15
Salt mixture (Osborne and Mendel)	4
Lard	8
Cod liver oil	2

The vitamin A method used is that approved by the U.S.P. X Revision Committee, and the vitamin B method that of Chase and Sherman ('31). The vitamin D method used is that of the U.S.P. X Revision Committee in which the new

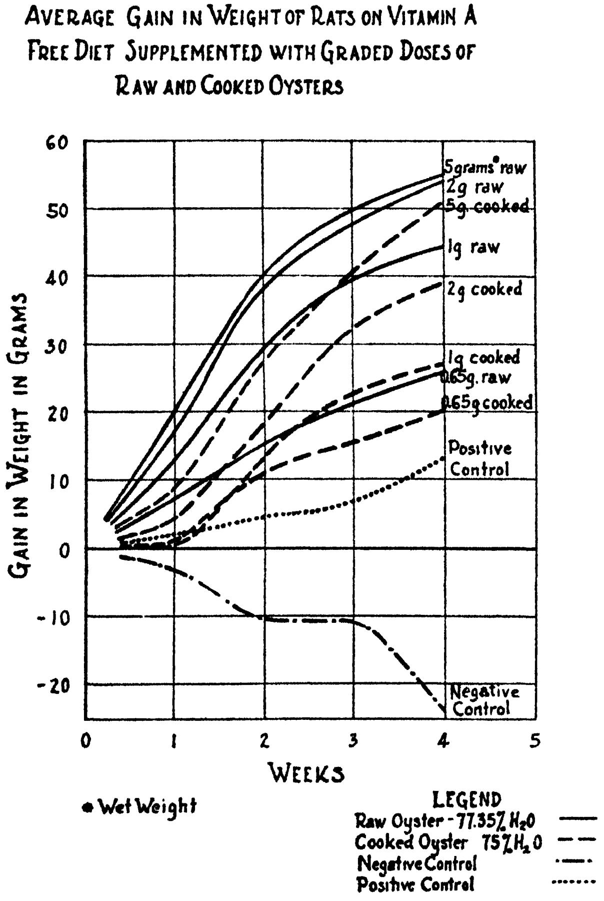

Figure 1

international irradiated ergosterol is used as standard. Vitamin D potency is expressed in vitamin D units U.S.P. (1934 revision) in which 1 unit is the antirachitic potency of 1 mg. of the standard solution. One of these units is approximately equivalent to 0.37 Steenbock units.

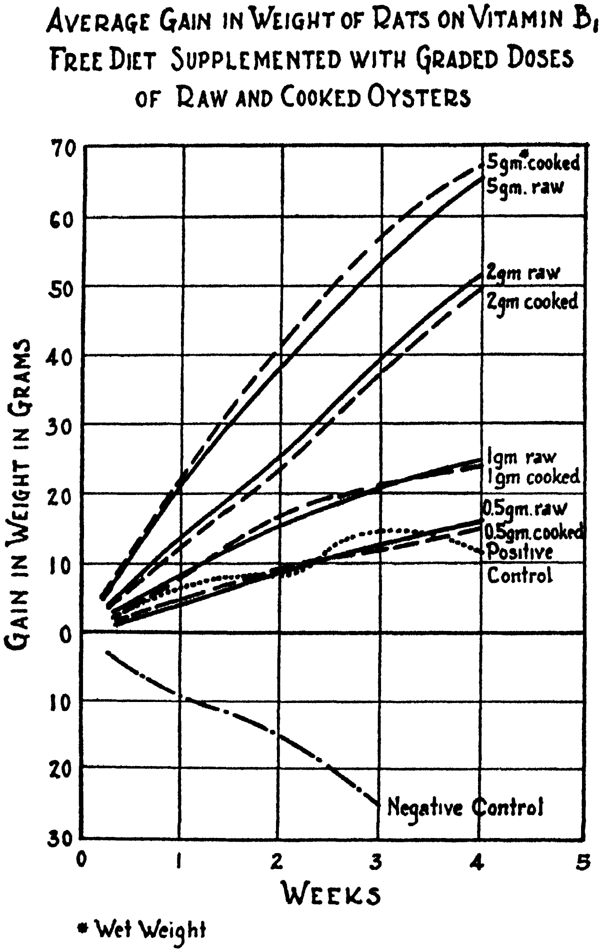
AVERAGE GAIN IN WEIGHT OF RATS ON VITAMIN B_1 FREE DIET SUPPLEMENTED WITH GRADED DOSES OF RAW AND COOKED OYSTERS

Figure 2

RESULTS

Figure 1 shows the data concerning vitamin A. The smallest dose of raw oyster given (0.65 gm.) permitted an average gain of 26.2 gm. in the 4-week period, a little more than twice the standard gain allowed for 1 unit of the vitamin. On this basis 1 gm. of oyster contained approximately 3 units of vitamin A. At all levels of feeding the cooked oyster was slightly lower in vitamin A content than the raw; 1 gm. of cooked oyster contained approximately 2.3 units of vitamin A.

Values for vitamin B(B_1) are given in figure 2. The lowest level of feeding in this experiment permitted an average gain of 16 gm. in the 4-week period, only very slightly more growth than that allowed for 1 Sherman unit of the vitamin. It can be said that 1 gm. of oyster contains approximately 2.0 units of vitamin B(B_1). The cooking had practically no effect upon the vitamin B(B_1) content.

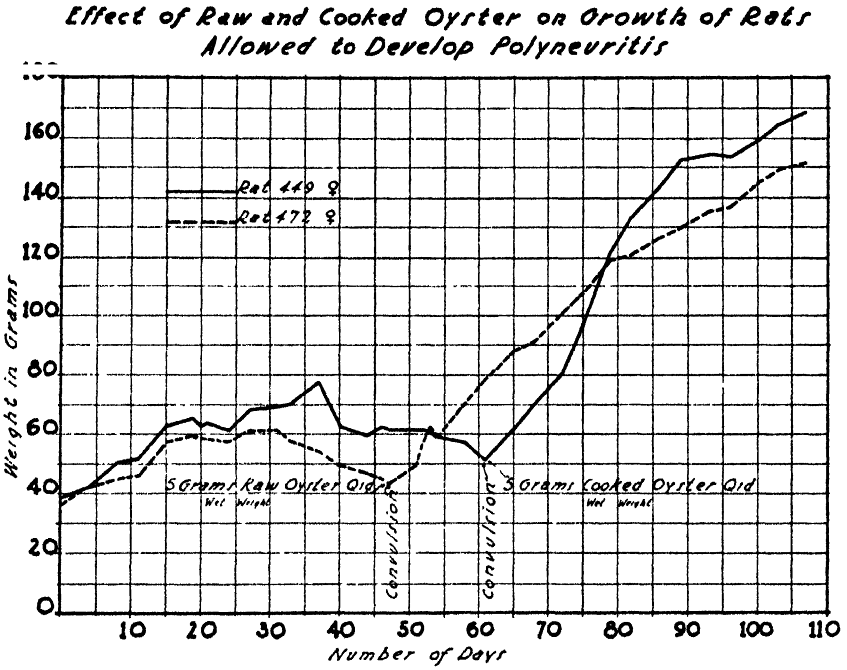

Figure 3

Two rats were permitted to develop severe beri-beri. During the height of the disease, when the animals were suffering from severe neuro-muscular disturbances, they were given 5 gm. of raw oyster and 5 gm. of cooked oyster, respectively. The results are given on figure 3 and show that both supplements caused an immediate gain in weight which was accompanied in each case by a rapid disappearance of the symptoms and a return to normal.

TABLE 2

Line tests on radii of rats fed raw oyster

SUPPLEMENT	DOSE	RAT NO.	LINE TEST	AVERAGE LINE TEST
	In gm.			
Raw oyster	5	1056	0	
	5	1063	0	0
	5	1070	0	
	5	1057	0	
	5	1064	0	
Raw oyster	10	1112	1+	
	10	1094	1+	1
	10	1135	1+	
	10	1120	1+	
	10	1055	1+	
Raw oyster	15	1115	2+	
	15	1095	2+	2.5
	15	1100	3+	
	15	1123	3+	
	15	1097	3+	
Raw oyster	20	1114	4+	
	20	1096	3+	3.8
	20	1116	4+	
	20	1111	4+	
	20	1099	4+	
	In cc.			
Olive oil	1	1121	0	
	1	1136	0	0
	1	1139	0	
	1	1091	0	
	1	1098	0	
Standard irradiated ergosterol 1: 10	0.02	1080	4+	
	0.02	1051	4+	4.0
	0.02	1072	4+	
	0.02	1058	4+	
	0.02	1073	4+	

Table 2 shows the data on the line tests for vitamin D. It was necessary to feed doses of 20 gm. in order to obtain healing equal to that of the one unit of irradiated ergosterol. This is a large amount of supplement to feed a rat and hence the accuracy of the test was not great. However, the test showed that these oysters contained approximately 1 vitamin D unit (U.S.P.) per 20 gm. of oyster or 0.05 unit per gram.

TABLE 3

Units of vitamins A and D in whole oyster and in oyster oil

	UNITS PER GRAM OF TOTAL OYSTER	UNITS PER GRAM OF OYSTER OIL
Vitamin A	3.0	208.0
Vitamin D	0.05	3.4

DISCUSSION

Whole oyster was found to contain approximately 3 U.S.P. units of vitamin A per gram and 0.05 U.S.P. unit of vitamin D. If all the fat-soluble vitamins are assumed to be in the fat of the mollusk, oyster oil is a rich source of vitamin A, though a poor source of vitamin D. The oysters used in these experiments contained 1.45 per cent fat, or 1 unit of vitamin A per 0.0048 gm. of fat, or 208 units of vitamin A per gram of oyster oil, and 1 unit of vitamin D per 0.29 gm. of fat, or 3.4 units of vitamin D per gram of oyster oil. The oyster seems able to concentrate vitamin A to nearly the same degree as the cod, but it is unable to store comparable quantities of vitamin D. Possibly the shell, the exo-skeletal structure of the mollusk, is laid down through quite a different mechanism from that of the vertebrate fishes, and it is conceivable that vitamin D plays either no role, or at least a different one from that which it plays in animals with an internal skeletal system.

The oysters used for these experiments were removed from the water in October, which is not a time when the oyster is actively depositing shell. Since a marked seasonal variation exists in the calcium metabolism of the oyster, it is possible that there might likewise be an annual cycle in vitamin D content. Likewise, the relatively large amount of vitamin A may have some relation to the approaching period of hibernation.

In stating units of vitamin D per gram of food substance, considerable caution must be exercised. The rat responds to the antirachitic action of oysters as compared with standard irradiated ergosterol in such a way that it takes 20 gm. of oysters to equal 0.02 cc. of 1:10 dilution of standard irradiated ergosterol. We are not, however, justified in concluding that a child would require the same relative number of oyster units of vitamin D as a rat. Children and rats apparently respond very differently to vitamin D from varying sources (Hess and Lewis, '33), and, therefore, we can draw no conclusion concerning the antirachitic potency of oysters for children.

The oysters suffered very little loss in vitamin potency during cooking. This was partly due, no doubt, to the fact that the oysters were cooked whole and ground for feeding after cooking. The vitamins are probably present largely in the visceral parts of the oyster and are therefore protected from the surface oxidation which takes place during exposure to cooking temperatures.

It is difficult to make exact comparison of these studies with those of Jones, Murphy and Nelson ('28) on the same subject, because the technic of vitamin assays has been further standardized since their studies were made. They stated that 2 gm. of raw oyster (0.32 gm. on a dry basis) furnished sufficient vitamin A to cure rats of xerophthalmia. These rats gained approximately 35 gm. This is about three times the gain allowed for 1 unit of vitamin A, which signified that the oysters used by the Washington workers contained approximately 1 unit of vitamin A per 1.5 gm. wet weight of oyster. This is approximately half the potency of the oyster used in the present studies. The difference may be due to age of the oyster, the type of food, and the season at which it was removed from the water. (The material used by Jones, Murphy and Nelson was Chesapeake Bay oysters obtained during the winter from the Washington market.)

The oysters used by Jones, Murphy and Nelson contained almost twice as much vitamin D as those described in these studies. This difference may have been due to the same factors as those which caused the differences in vitamin A potency.

SUMMARY

The vitamin A content of oysters taken from the beds in October was found to be approximately 3 U.S.P. units per gram. Assuming that all of the vitamin A is in the fat of the oyster, oyster oil contained 208 units per gram. There was a slight destruction of vitamin A during cooking.

The vitamin D content of oysters harvested in the fall was found to be approximately 0.05 U.S.P. unit per gram. Assuming that all of this vitamin is also in the fat of the oyster, the oyster oil contained 3.4 units per gram. The discrepancy between rat units of vitamin D and antirachitic potency for children is emphasized.

The vitamin $B(B_1)$ content of oysters was found to be approximately 1.5 Sherman units per gram. There was no appreciable destruction during cooking.

Oysters were found to be an excellent food source of vitamin $B(B_1)$, a relatively good one of vitamin A, and a very modest source of vitamin D.

Whether the vitamin content of oysters varies at different times in the year remains to be determined.

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MINERAL EXCHANGES OF MAN

IV. VARIATIONS IN THE MINERAL CONTENT OF DIETS WITH A CONSTANT RAW WEIGHT FORMULA [1]

SAMUEL H. BASSETT AND HELEN E. VAN ALSTINE

Department of Medicine, University of Rochester School of Medicine and Dentistry and Medical Clinic of the Strong Memorial Hospital, Rochester, New York

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The present communication is intended to serve as a supplement to the first paper in this series (Bassett, Elden and McCann, '31). It deals specifically with the application of a method for approximate quantitative determination of the amount of mineral elements ingested by human subjects during the course of protracted balance experiments. A brief review of the usual methods employed in the estimation of mineral intake is included, since it is pertinent to the present discussion.

1. Estimation of mineral content of food by use of tables of averages

The amount of each of the elements in the diet may be calculated from the raw weights of the different foods ingested by use of average values for such elements as found in the literature. Tables showing these average values have been compiled by Sherman ('26), Rose ('31) and others. The method serves a useful purpose when it is unnecessary or impossible to obtain a more precise estimate of the mineral content of the food ingested.

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[1] Aided by a grant from the Fluid Research Fund contributed by the Rockefeller Foundation.

2. *Estimation of mineral content by analysis of food*

a. Actual analyses of the various food stuffs to be used in the diet may be made by the investigator and the values thus obtained employed in calculating the intake. Such a method is capable of giving excellent results when the total number of items on the menu is relatively small and when sufficient quantities of the raw foods may be purchased in advance to insure an adequate supply for the duration of the experiment. It is obviously important that the food be of as uniform a composition as possible and that the source of the individual items be constant. The wide variations in the mineral content of grain grown under different conditions of soil and climate (Greaves and Hirst, '29) serve to emphasize this point. In general, the method would seem more suitable for metabolism experiments on animals or small children where a monotonous diet may be comparatively well tolerated.

b. One or more sample diets duplicating in all respects the diet or diets to be ingested by the subject of the experiment in a metabolic period are prepared, the several items mixed, dried and pulverized. Samples from the composite mixture are then weighed out, ashed and analyzed. The total of each of the mineral constituents of a day's food are computed from these analyses. Complete duplicate analyses of the food ingested in each subsequent metabolic period are not undertaken, but the formulae of the diets to be used are made, in so far as possible, both qualitatively and quantitatively identical with those which have been analyzed. It, therefore, appears logical to employ the values which were derived from the original analyses in making calculations of mineral intake in metabolic periods which follow. This method has been used by Salter, Fulton and Angier ('31) in the calculation of the acid-base content of diets and has also been employed in some of our metabolic work (Bassett, Elden and McCann, '31). It has become increasingly apparent, however, that even when the original menu is accurately duplicated in preparing successively the same mixtures of foods for analysis, the analyses may reveal considerable period by period variability in the mineral content of the diet.

c. The third method in general use is laborious, but seems to insure greater accuracy. An aliquot portion of each item of food is weighed or measured at the time the diet is prepared and the aliquots are saved for analysis. The samples saved may conveniently be pooled for a day or for several days when the periods are longer. The intake is computed from analysis of the pooled samples which are subjected to the same analytical methods as are used in obtaining the mineral content of the excreta. Technical details of a modification of this procedure are described in subsequent paragraphs.

EXPERIMENTAL

Composition of diets and length of periods

Two diets were chosen for their high and low calcium content, respectively. Tables 1 and 2 give the daily menus of these diets and the total quantity of each item of food given during a 6-day period. Collection periods were fixed at 6 days, since a period of this length has been used most frequently in our metabolism experiments. The high calcium diet (table 1) was sampled for eleven periods (66 days) and the low calcium diet (table 2) for ten periods (60 days).

Method of sampling

A sufficient quantity of raw food materials was assembled to meet the menu requirement for the day, plus an amount which permitted sampling. As each item was weighed or measured for the patient, an aliquot part equal to one-quarter the amount of the serving was saved for analysis. These aliquots were chosen so that they represented as nearly as possible the actual food received by the patient. Samples were transferred from the receptacle in which the measurement was made to a large cylindrical Pyrex glass collection jar. Solid foods were minced prior to transfer. The jar was kept covered and in a refrigerator when not in use. When meat was served it was ground, mixed, and portions weighed from the ground mixture. The aliquot saved for analysis was cooked in a manner identical to that of the patient's serving (Bassett, Elden and McCann, '31), then added to the pool. When a custard or other dish requiring the addition of several items was served, the raw food stuffs were assembled and the weight or volume of each item increased by an amount equal to one-quarter of the weight or volume

TABLE 1

High calcium diets. Raw food constituents (edible portions)

FOOD	RAW MEASURE—GRAM OR CUBIC CENTIMETER						TOTALS FOR 6 DAYS
	Day of period						
	1st	2nd	3rd	4th	5th	6th	
Milk	580	750	620	780	720	520	3970
Cheese	30		30			30	90
Egg	220	210	130	190	190	200	1140
Bread	40	40	40	40	40	30	230
Flour	5		5				10
Butter (salted)	15	20	20	25	20	15	115
4-X cream	30	35	30	35	60	65	255
D-Zerta powder		3.5	3.5		3.5		10.5
Carrots	60						60
Tomatoes		100	100		100	100	400
Onions	80	80		80			240
Peas			60			60	120
Squash			100				100
Spinach				80	80		160
Orange juice	400	500	350	500	400	300	2450
Apple	100		100			100	300
Banana		40	40	40	140		260
Prune						20	20
Sugar	15	5	5	10	15	15	65
ESTIMATED DAILY TOTALS OF							
Protein	64.9	62.6	61.2	59.6	61.2	63.0	372.5
Carbohydrate	140.6	139.2	145.6	144.4	154.3	129.1	853.2
Fat	83.6	84.6	80.9	87.8	91.9	93.1	521.9
Calories	1626	1614	1602	1655	1737	1652	9886

Maximum number of items per diem, 14.
Minimum number of items per diem, 10.
Total number of items used, 19.

which the patient was to receive. After the necessary cooking, the total weight of the finished product was obtained in grams and this value divided by five. Since one-fifth of the total weight was equivalent to one-fourth of the amount actually given the patient, that amount was weighed as an aliquot and added to the pool. Obviously, only such items

TABLE 2

Low calcium diets. Raw food constituents (edible portion)

FOOD	RAW MEASURE—GRAM OR CUBIC CENTIMETER			TOTALS FOR 6 DAYS
	Day of period			
	1st and 4th	2nd and 5th	3rd and 6th	
Tenderloin	220		220	880
Round steak (as Hamburg)		75		150
Pork chops		120		240
Bread	40	20	20	160
Rice	20	10		60
Oatmeal		15		30
Farina			15	30
Potato	100	100	200	800
Cucumber	50		50	200
Tomato	100	140	160	800
Lettuce	40	40	40	240
Celery		20	20	80
Onion		20		40
Banana	100	100	50	500
Pineapple	100	100	150	700
Apple	100	100		400
Grape juice	100	200	100	800
Butter (salt free)	20	25	20	130
Mayonnaise	25	50	25	200
40 per cent cream	80	40	110	460
Walnuts			15	30
Sugar	25	15	20	120
Honey (strained)	50	30		160
ESTIMATED DAILY TOTALS OF				
Protein	49.1	50.0	52.8	303.8
Carbohydrate	200.9	192.5	144.3	1075.4
Fat	130.5	131.2	151.8	827
Calories	2243	2215	2221	13,358

Maximum number of items per diem, 19.
Minimum number of items per diem, 16.
Total number of items used, 23.

may be compounded in this way as will give a homogeneous mixture when cooked. An illustration may serve to clarify the apparent complexity of the procedure. Let it be assumed that the items used in the preparation of ice cream were:

	For patient	*For analysis*	*Total*
Milk	100.0 cc.	25.0 cc.	125.0 cc.
Egg	37.5 gm.	9.4 gm.	46.9 gm.
Sugar	6.2 gm.	1.5 gm.	7.7 gm.
Cream—40 per cent	25.0 cc.	6.2 cc.	31.2 cc.

The total for each item as given in the third column of the tabulation was the amount actually used in preparation of the ice cream. After cooking and freezing, the total weight of the assembled items, exclusive of the small amounts which had necessarily adhered to cooking utensils, was found to be 198 gm. From this net weight 39.6 gm. were saved for analysis and the remaining 158.4 gm. were served to the patient.

When the final meal of the period had been sampled, the contents of the collection jar were thoroughly mixed by stirring with a glass rod and the jar placed in an electric drying oven. The temperature of the oven was regulated so that it remained between 90 and 95° C. and a current of air was slowly drawn through it during the process of drying, thus preventing the condensation of any considerable amount of moisture upon its inner wall. As drying progressed, the diet sample was stirred from time to time to ensure as homogeneous a composition as possible. No attempt was made to dry to a constant weight, but after 3 or 4 days when the food seemed to be thoroughly dry, the sample was removed from the oven, pulverized in a porcelain mortar and returned to the original container. The gross weight of sample and container to 0.1 gm. was obtained and the powdered diet immediately transferred to a rubber stoppered glass bottle of suitable size where it was protected from the changing moisture content of the air of the room. The actual weight of the sample was obtained by difference after weighing the carefully washed and dried container.

Analytical methods

Large samples (60 to 70 gm.) were accurately weighed from the dry, pulverized diet and ashed in an electric muffle.[2] A solution of the ash in dilute hydrochloric acid was used for the determination of sodium, potassium, calcium, magnesium and phosphorus. Duplicate ashings were made only when the analysis of the original ashing gave an exceptionally high or low result. In such instances the questionable values were verified by repeating the analytical procedure using a duplicate ashing. The method of analysis, with the exception of the methods used in determination of potassium and chlorine and certain modifications of the iron method, has been described elsewhere (Bassett, Elden and McCann, '31). In the case of potassium, a gravimetric procedure (Van Alstine and Bassett, '34) was substituted in place of Shohl and Bennett's ('28) micro-volumetric method. Chlorine was determined by the method of Birner ('28).

Iron. Special care was taken to avoid contamination of the sample with iron. Approximately 100 gm. of the dried material were accurately weighed and ashed in an electric muffle at a temperature not exceeding 600° C. The ash was dissolved in a solution of pure hydrochloric acid diluted one-half with distilled water. After digestion for several hours on the steam bath to insure complete solution of the iron, the carbon residue was removed by filtration, washed and reashed. An extract of the ash thus obtained was made with hydrochloric acid and added to the original solution, which was finally concentrated and made to a volume of 100 ml. Twently ml. aliquots, containing 0.5 to 0.9 mg. of Fe, were pipetted into 100 ml. Florence flasks and further concentrated to a volume of 8 to 10 cc. When the contents of the flasks had cooled, two or three glass beads and 10 cc. of concentrated sulphuric acid were added to each. (The sulphuric acid was added while the neck of the flask was grasped by a small pair of crucible tongs and held in an inclined position. If this precaution is not taken, the reaction between the added acid and the contents of the flask may be so violent as to cause loss of material.) Each flask was then heated to boiling over a free flame while held in the inclined position and agitated sufficiently to prevent bumping. With the beginning evolution of fumes of sulphuric acid the heat was increased and the process continued until all but 2 or 3 ml. of sulphuric acid had been expelled.[3] Finally 30 ml. portions of 20 per cent sulphuric acid were added to the contents of each flask, the mouths covered with a watch crystal and the flasks placed on the steam bath for 6 hours.

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[2] The temperature of the electric muffle used in the preparation of ash attained a maximum of 600° C. This is a somewhat higher temperature than is usually recommended when sodium or potassium is to be determined in ash (Peters and Van Slyke, '32).

Boiling with concentrated sulphuric acid was found to insure complete oxidation of the iron without addition of any oxidizing agent likely to interfere with the subsequent titration. In the final stages of concentration of the solution some ferric sulphate and calcium sulphate precipitated. The ferric sulphate was soluble with some difficulty in 20 per cent sulphuric acid, hence the long period of digestion on the steam bath. Reference to the actual method of titration has been given in the first paper (Bassett, Elden and McCann, '31).

Analysis of data

Dunn ('29) has shown that statistical methods may be applied effectively to even as small a series of observations as were obtained in these period by period analyses of the food, provided that the limitations of such an application be kept in mind. Table 3 presents in a condensed form the result of such statistical analysis as the data appear to warrant. The mean, its standard deviation, and coefficient of variation have been computed for each element determined in the two series of diets. Calcium and iron are the mineral constituents showing the greatest variation in each series. The least variable element is potassium, as found in the low calcium diet. In table 4 the means determined by analysis are compared with those calculated from Sherman's tables. In the case of the high calcium diet, one notes that the values of nitrogen, sodium, potassium and magnesium, as determined by analysis, are in fair agreement with their calculated values, while the amounts of calcium, phosphorus, chlorine and iron determined differ considerably from the amounts calculated. The means of the series of analyses made of the low calcium diet agree fairly well with the calculated values in the case

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[3] The digestion with sulphuric acid must be performed in a well-ventilated hood.

TABLE 3

Mean values of mineral constituents of high and low calcium diets calculated from period by period analysis of the food

ELEMENT	HIGH CALCIUM DIET[1]			LOW CALCIUM DIET[2]		
	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
	Grams in 6 days	Grams	Per cent	Grams in 6 days	Grams	Per cent
Nitrogen	62.16 ±0.394	1.85	2.9	55.97 ±0.296	1.32	2.3
Sodium	6.89 ±0.047	0.22	3.2	3.37 ±0.018	0.08	2.3
Potassium	17.74 ±0.13	0.61	3.4	15.52 ±0.029	0.13	0.8
Calcium	7.01 ±0.11	0.53	7.5	0.91 ±0.018	0.08	8.8
Magnesium	1.363 ±0.007	0.036	2.6	1.178 ±0.007	0.033	2.8
Chlorine	11.10 ±0.08	0.40	3.7	6.07 ±0.083	0.37	6.1
Phosphorus	8.61 ±0.09	0.44	5.1	4.28 ±0.029	0.13	3.0
Iron	0.0514±0.0008	0.0039	7.6	0.0577±0.0011	0.0049	8.6

[1] Each mean is calculated from the results of analysis of food in eleven consecutive metabolic periods.

[2] As above, except that in this instance there were only ten consecutive metabolic periods.

TABLE 4

Mean values of mineral constituents computed from analysis of food are compared with mean values computed by use of Sherman's tables

ELEMENT	HIGH CALCIUM DIET			LOW CALCIUM DIET		
	Mean found by analysis	Mean calculated from Sherman	Difference between mean found and calculated	Mean found by analysis	Mean calculated from Sherman	Difference between mean found and calculated
	Grams in 6 days	Grams in 6 days	Per cent	Grams in 6 days	Grams in 6 days	Per cent
Nitrogen	62.16	59.68	+ 2.4	55.96	48.64	+15.0
Sodium	6.89	6.80	+ 1.3	3.37	3.55	— 5.0
Potassium	17.74	17.65	+ 0.5	15.52	18.00	—13.8
Calcium	7.01	7.76	— 9.7	0.91	1.35	—32.6
Magnesium	1.363	1.313	+ 3.8	1.179	1.170	+ 0.77
Chlorine	11.10	9.99	+11.2	6.07	5.83	+ 4.1
Phosphorus	8.61	7.81	+10.2	4.28	4.55	— 5.9
Iron	0.0514	0.0676	—24.0	0.0577	0.0653	—11.6

of sodium, magnesium, phosphorus and chlorine. All others show a difference between found and calculated means which is greater than 10 per cent.[4]

An approximation of the excess of acid or base in the ash of the two diets (derived from the mean values of acidic and basic constituents) is given in tables 5 and 6. From these it appears that the ash of the high calcium diet is alkaline, and of the low calcium diet acid. However, attention must be called to the fact that the sulphur content of the food was computed from tables of averages (Sherman, '26).

TABLE 5

Acid-base components of high calcium and phosphorus diet, milliliters of 0.1N acid and base per 6-day period

BASE	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	ACID	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	EXCESS BASE ML. 0.1N SOLUTION
Na	6.89	2995	Cl	11.10	3130	
K	17.74	4537	S[1]	4.81	3000	
Ca	7.01	3505	P[2]	8.61	4999	
Mg	1.363	1120				
Totals		12,157			11,129	1028

[1] Mean value for sulphur calculated from Sherman.

[2] Base equivalent of phosphorus calculated to pH7.4 (ml. 0.1M $H_3PO_4 \times 1.8$).

TABLE 6

Acid-base components of low calcium diet milliliters of 0.1N acid and base per 6-day period

BASE	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	ACID	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	EXCESS ACID ML. 0.1N SOLUTION
Na	3.37	1465	Cl	6.07	1711	
K	15.52	3969	S[1]	5.83	3646	
Ca	0.91	455	P[2]	4.28	2484	
Mg	1.178	968				
Total		6857			7841	984

[1] Mean value for sulphur calculated from Sherman.

[2] Base equivalent of phosphorus calculated to pH7.4 (ml. 0.1M $H_3PO_4 \times 1.8$).

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[4] These results do not detract from the general usefulness of Sherman's tables. Differences between means found and calculated would probably be as great if any other set of average tables were substituted in their place.

The difference in each case is about 1 liter of 0.1N base or acid. If this difference is due to error introduced by estimation of the sulphur content of the food, and if it be assumed, for example, that the diets actually had a nearly neutral ash, then in the first instance (table 5) the calculated value assigned to sulphur is about 33 per cent too low and in the second instance (table 6) about 27 per cent too high. While the actual sulphur content of the food may vary from the calculated to the extent indicated (see table 4 for examples), such a variation is probably unlikely. It is apparent, however, that no definite statement regarding the acidity or alkalinity of the ash of these diets may be given without more accurate knowledge of their sulphur content.

DISCUSSION

Number of items on menu

Reference to tables 1 and 2 shows that nineteen different foods were used in the high calcium diet and that twenty-three foods were used in the low calcium diet. Granting that simplicity in a diet is desirable for metabolic work, these diets appear to include an unnecessarily large number of items. On the other hand, the effect of an extremely monotonous diet on the subject of the experiment must be considered. Such a diet may be comparatively well tolerated by children, but when given to adults frequently leads to refusal or smuggling of food.

The importance of good cooking and attractive menus has been emphasized by Gephart and Du Bois ('15). With the diet rigidly limited to four or five foods, the serving of attractive well-cooked meals becomes a serious problem, if not an impossibility. The fact that the menus listed in table 1 were, in a certain instance, repeated every 6 days for approximately 4 months and were well tolerated is an argument in favor of a liberal diet.

Evaluation of technical errors

The following experiment was performed in order to determine whether the failure to obtain uniform results in period by period sampling of the food depended upon errors inherent in the methods employed or was due to actual variations in the mineral content of the foods served in these periods.

Unused portions of the dried samples of food from the high calcium diet were pooled and thoroughly mixed together. When the mixture appeared to have become neither more nor less homogeneous than the dried food sample of a single period, ten consecutive portions of 60 gm. each were weighed out and ashed. The ten samples of ash thus prepared were subjected to the routine methods of analysis. In addition, twenty smaller samples of 1 gm. each were used for the determination of chlorine. The data obtained have been summarized in table 7.[5] Strictly speaking, these data can be compared only with the period by period analyses of the high calcium diet (table 3), but it is reasonable to suppose that consecutive analyses of a similar specimen of food prepared from the low calcium diet would yield comparable results. From the tabulation it is evident that the standard deviations

TABLE 7

Consecutive analyses of a single specimen of dried food showing variations due to technical errors

ELEMENT	NUMBER OF SAMPLES ASHED	WEIGHT OF EACH SAMPLE IN GRAMS	MEAN IN GRAMS[1]	STANDARD DEVIATION IN GRAM	COEFFICIENT OF VARIATION
Sodium	10	60	7.09 ±0.021	0.096	1.36
Potassium	10	60	18.42 ±0.068	0.305	1.65
Calcium	10	60	6.87 ±0.015	0.066	0.96
Magnesium	10	60	1.388±0.007	0.033	2.37
Chlorine	20	1	11.11 ±0.046	0.296	2.66
Phosphorus	10	60	8.46 +0.031	0.136	1.61

[1] Each mean has been computed on the basis of a 6-day period.

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[5] A statistical evaluation of the amounts of nitrogen and iron in consecutive samples taken from the same specimen of food has not been made.

are, for the most part, smaller than those found in the period by period analysis of the food. Comparatively large fluctuations in the amounts of calcium and phosphorus no longer occur. The results obtained in the cases of sodium and potassium are somewhat less convincing, although when compared with the previously cited data (table 3) for the high calcium diet it is found that the respective standard deviations have been decreased about 50 per cent. Magnesium, on the other hand, showed nearly as much variability in the series of analyses of the single specimen of dried food as was found in period by period analysis. Chlorine showed more variability than any of the other elements. However, previous experience leads us to attribute much of the variation in this instance to lack of uniformity among small samples. While the data do not permit a differentiation between errors introduced by sampling and those introduced by analytical methods and manipulations, they show the gross technical errors exclusive of errors which may be introduced in the diet kitchen. Unfortunately, the latter are not readily susceptible to statistical measurement. Since we have been unable to detect any gross inaccuracy in the initial measurement of samples of food by the dietitian, the assumption has been made that the larger deviations of sodium, potassium, calcium and phosphorus from their respective means (as found in period by period analysis of food) actually represent fortuitous variations in the concentrations of these elements in the food of different periods. If this is so and one is to avoid errors of unknown and perhaps considerable magnitude, it becomes necessary to sample and analyze the food given in every metabolic period. When the same diet formula is repeated many times in consecutive metabolic periods, and analyzed in each by the methods described, mean values of chlorine, magnesium and probably of nitrogen may be computed, and these values used as an approximation of the true intake in each period. Where calcium, sodium, potassium and phosphorus are concerned, it seems advisable to compute the intake in each period from analysis of the sample of food collected in that period.

The limited data available in the case of iron do not permit us to submit statistical evidence from which a probable differentiation can be made between technical errors and actual changes in the iron content of the food.

SUMMARY

1. A method for determination of mineral intake in human metabolic experiments has been described in detail.

2. The method has been applied to determine the amount of nitrogen, sodium, potassium, magnesium, calcium, phosphorus, chlorine and iron in, a) high calcium diet, b) low calcium diet.

3. The mineral content of the high calcium diet was determined during eleven consecutive metabolic periods of 6 days each, and of the low calcium diet during ten consecutive periods of 6 days each.

4. Statistical methods have been employed in an attempt to determine the magnitude of the technical errors of the procedure and to differentiate between such errors and actual variations in the mineral content of the food mixture.

5. The implications of the statistical analysis of the data have been discussed in the text.

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THE RELATION OF MANGANESE TO CONGENITAL DEBILITY

AMY L. DANIELS AND GLADYS J. EVERSON
Iowa Child Welfare Research Station, State University of Iowa, Iowa City

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The high percentage of deaths among the young of rats receiving milk fortified with copper and iron, in comparison with the vigor and physical well-being of the young of rats receiving the same diet to which manganese was added has led to an inquiry concerning the cause of the infant mortality among the former group. Do the young die of congenital debility, the result of too little manganese in the diet of the mother during gestation, or is there a failure in lactation due to the lack of mammary gland development, as suggested by Orent and McCollum ('31)?

The hypothesis of a vitamin B deficiency as the cause of death of the young of milk-fed rats, postulated by Sure ('24, '29) would seem to be untenable, since rats receiving milk to which copper, iron and manganese were added in adequate amounts have been raised through five generations.[1] The vigor and weaning weights of the young would seem to attest to the efficiency of the diet. The explanation of the beneficial effect of the larger amounts of vitamin B extracts and concentrates used by Sure in lactation experiments with rations consisting of 50 per cent milk powder would seem to be either contamination with manganese of the materials tested or considerable destruction of vitamin B in the milk powder, since the nutritive ratio of the milk powder ration used by Sure is approximately the same as that of Guernsey milk in our experiments and therefore the B_1 of the two rations should be similar. It is interesting to note that Sure found a 25 per cent alcoholic extraction of wheat embryo much more potent as a source of vitamin B for lactation than a 75 per cent extraction. It is probable that the 25 per cent extract contained significant amounts of manganese.

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[1] Unpublished data.

The importance of manganese in nutrition, more especially in relation to reproduction, has been demonstrated by Orent and McCollum ('31), Skinner, Van Donk and Steenbock ('32) and Kemmerer, Elvehjem and Hart ('31). Too little manganese results in testicular degeneration and sterility after about the 150th day. Young born of females fed manganese-free diets die either at birth or shortly after. Spectrographic analysis of the ash of the young indicates that the manganese is in low concentration and suggests that the young were not 'well-born.' On the other hand, the mothers of these manganese-low young manifest little interest in their offspring, giving them scant opportunity to suckle. Examination showed little mammary gland development (Orent and McCollum, '31). The young, therefore, may have obtained insufficient nourishment. In order to test this point, Orent and McCollum transferred young of mothers fed manganese-free diets to stock mothers and young of stock mothers to the manganese-free mothers. Of the 107 young transferred to stock mothers, only one was raised, with a weaning weight much below the average. In two instances stock young given to mothers receiving manganese-free diets were slightly more successful. One mother raised two stock young of the seven which were substituted for her own; and the other, five out of seven. The weaning weights of these conformed to the average of stock young. The failure of the stock animals to raise the young of manganese-low mothers was the more notable, since, in general, lactating rats are exceedingly open-minded on the subject of adoption and will accept and suckle young of other animals with little question.

Orent and McCollum's results, tentatively interpreted as indicating failure in lactation of the manganese-low mothers, seemed rather to point to congenital debility of the young, or possibly to too little vitamin B in the ration used. In order to test this point with milk-fed animals, the investigation herein reported was undertaken.

EXPERIMENTAL PROCEDURE

Two groups of animals, one at 11 days and a second at 8 weeks of age, consisting of four females and two males in each group, were transferred from stock to the experimental laboratory and given a ration consisting of boiled Guernsey milk to which were added 0.8 mg. of copper, 5 mg. of iron and 2 drops of 2 per cent potassium iodine solution to every 100 cc. of milk. For purposes of comparison other groups were tested: one, a fourth generation to receive the milk, copper and iron ration supplemented with 1.5 mg. of manganese per 100 cc. of milk; and others, consisting of animals receiving our stock ration with iron, copper and manganese additions.[2] When the young of these various groups were born, one of two procedures was followed: 1) The young shortly after birth were prepared for analysis of the manganese content. 2) The young were exchanged, manganese-low young to stock mothers and stock young to manganese-low mothers, thus giving an opportunity to determine whether it is possible for the mothers receiving the milk, copper and iron diet to suckle vigorous stock young or for the manganese-low young to survive if sufficient food is available. Theoretically, the quality of the milk produced by the respective mothers should not differ in the amount of manganese contained therein, since, according to Kemmerer and Todd ('31, '32), it is not possible to increase the manganese content of milk by feeding. Under normal conditions, the manganese content of cow's milk is low, differs with the individual, and cannot be increased by feeding. Nothing is known directly regarding the manganese content of rats' milk. It seems probable, however, that similar factors control milk production in both species.

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[2] The stock ration consisted of a mixture of dried cooked soy beans, yellow corn, sodium chloride and calcium carbonate. Pasteurized Holstein milk with 0.4 mg. of Cu, 0.75 mg. of Mn., 2 mg. of Fe and 1.5 mg. of I per 100 cc. was also given ad lib.

In the lactation tests six of a given litter were transferred to the foster mother as soon after birth as possible. The efficiency of the mother as a milk producer was judged by the number of young successfully suckled, the weaning weight of the young, and the weight gain or loss of the mother during lactation. A record of the milk consumption of the mothers receiving the two milk diets was kept in order to determine if failure in lactation was caused by a possible low food ingestion. The physiological well-being of the young at birth was judged by the viability and the gain in weight during the suckling period.

In order to obtain further information regarding the high mortality of the young of the milk-fed groups, comparisons were made of the manganese content at various ages from weaning to sexual maturity of animals receiving the milk ration with and without the manganese additions. For these tests, a few days after weaning, nine young were placed on the milk-copper-iron ration and nine on the milk ration with the manganese addition. Three of each group subsequently were taken for analysis at intervals of approximately 2 weeks, the milk-manganese animals being continued 2 weeks longer than the milk, copper and iron group.

In preparation for analysis, after anesthetizing all animals were washed with distilled water, the alimentary tracts were removed, the bodies were dried, subsequently ashed, and the manganese determined according to the method of Skinner and Peterson ('30). To obtain sufficient material for satisfactory tests with the newborn animals it was found necessary to use at least two litters; whereas with the older animals one animal was found sufficient. In two cases comprising the young of manganese-low animals, known amounts of manganese were added in order to obtain more satisfactory determinations and thus check the reliability of the results with other low manganese young.

Eight first generation females receiving the milk-copper-iron diet, produced in the time allowed at least 103 young; 47 in first litters, 33 in second, and 23 in third. All of the third litters and some of the second were sired by stock males. Since in some instances the young were born during the night, more may have been born than were seen; indeed, there was evidence that such was the case. The data, however, pertain only to such young as were intact. Of these, 41 either were born dead or died within two hours, 25 were anesthetized for subsequent manganese analysis, and 37 were transferred to 7 stock mothers. Sixty-nine per cent of these subsequently died within a few days; 12 were raised by 2 females (6 each) to weaning weights of 53.7 and 41.5 gm., respectively.

The mortality record of the young of these low-manganese females is quite different from that of the young of the fifth generation females receiving the milk diet with added manganese, or of the stock animals. In the milk-manganese groups, 100 per cent of the young were reared with an average weaning weight at 28 days of 69.2 gm., and in the stock groups 98.6 per cent were raised with an average weaning weight of 70.1 gm. (table 1).

The low-manganese mothers were more successful in suckling the young of stock than their own offspring (table 1). Eleven litters of six young each of stock mothers were given to mothers receiving the low-manganese diet. In five instances, these low-manganese mothers had lost all their young at birth; in five, part of the litter had died and the remainder which were given to stock mothers to raise, subsequently died. Four of the groups transferred from stock replaced first litters of the low-manganese mothers; four, second litters; and three, third litters. Notwithstanding the high mortality of the young of the low-manganese mothers, these animals were able to suckle successfully 96.9 per cent of their foster young to weaning weights (average 58.7 gm.) which compared favorably with those of the milk-manganese mothers and not far below those of the young of the stock mothers reported. There was little difference in the lactation tests

TABLE 1

Relation of manganese content of the diet to lactation

RAT NO.	AGE WHEN PLACED ON DIET	LITTER SEQUENCE	AGE OF MOTHER AT BIRTH OF YOUNG	NUMBER OF YOUNG BORN	WEIGHT AT BIRTH	VIABLE YOUNG	MN-LOW YOUNG ADOPTED BY STOCK	STOCK YOUNG ADOPTED BY MN-LOW MOTHERS	YOUNG RAISED	WEIGHT OF YOUNG 28 DAYS	WEIGHT GAIN OR LOSS OF MOTHER 28 DAYS
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol.+ 1.5 mg. Mn per 100 cc.											
			Weeks		*gm.*	*Per cent*	*Number*	*Number*	*Per cent*	*gm.*	*Per cent*
50	4th gen.	1	14.5	6	7.1	100	..	..	100	63.3	— 2.7
44	4th gen.	1	15.0	3[2]	6.8	100	..	..	100	76.0	— 3.9
45	4th gen.	1	14.8	5[3]	6.6	100	..	..	100	74.1	— 5.8
43	4th gen.	1	15.5	10	6.0	100	..	..	100	76.0	+ 1.3
42	4th gen.	1	15.9	9	4.8	100	..	..	100	56.6	— 4.5
Average			15.1	6.6	6.4	100	..	..	100	69.2	— 3.1
Diet: Stock food + manganese											
11	....	..	...	7	9.6	100	..	..	100	73.6	— 6.8
12	....	..	...	6	7.6	100	..	..	100	72.1	— 0.0
13	....	..	...	11	9.0	100	..	..	100	60.0	— 2.8
14	....	..	...	10	6.2	90	..	..	90	65.5	— 3.6
16	....	..	...	9	6.6	100	..	..	100	82.2	— 5.0
17	....	..	...	8	7.5	100	..	..	100	75.6	— 6.0
18	....	..	...	9	9.3	100	..	..	100	66.3	—11.0
Average				8.6	8.0	98.6	..	..	98.6	70.1	— 5.0
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol.											
1	8 weeks	1	14.0	7	4.8	0	..	..	...	...	...
2	8 weeks	1	17.0	9	5.1	[1]	..	6	66	58.5	— 7.6
3	8 weeks	1	13.0	7	3.8	43	3[2]	..	...	...	...
5	11 days	1	16.0	8	5.1	[1]	..	6	100	65.6	+ 2.7
6	11 days	1	16.0	3	5.3	0	..	..	...	...	...
7	11 days	1	18.0	5	6.8	0	..	6	100	60.7	+ 1.4
8	11 days	1	19.0	8	5.6	75	6[4]	6	100	56.5	+ 1.4
Average			16.1	6.7	5.2	...	..	...	91.5	60.5	— 0.52
1	8 weeks	2	20.0	7	5.3	85.7	6[2]	6	100	69.0	+ 5.7
2	8 weeks	2	22.0	8	4.9	[1]	..	6	100	49.2	— 2.7
5	11 days	2	27.0	5	5.0	0	..	6	100	49.0	—16.0[6]
6	11 days	2	21.0	9	5.0	77.7	7[2]	..	...	...	...
7	11 days	2	28.0	4	6.0	0	..	6	100	65.0	± 0.0
8	11 days	2	...	0	..	...	..	..	...		
Average			23.6	5.1	5.2	...	..	..	100	58.0	— 3.2
1	8 weeks	3	30.0	7	5.4	85.7	6[2]	6	100	60.7	+ 3.4
2	8 weeks	3	32.0	8	4.4	75	6[5]	6	100	51.8	+ 3.1
6	11 days	3	28.0	8	5.5	37.5	3[2]	6	100	60.1	— 2.7
Average			30.0	7.6	5.1	...	..	..	100	57.5	— 1.3

[1] Anesthetized.

[2] Died.

[3] Young of other milk-Mn groups added to make litter of six.

[4] Weaning weight 28 days—53.7 gm.

[5] Weaning weight 28 days—41.5 gm.

[6] Diarrhoea during excessive heat.

of the animals which were placed on the low-manganese diet at 11 days (animals 5, 6, 8, table 1) and 60 days (animals 1, 2, table 1) of age, respectively.

The manganese content of the young of stock (average 0.002 mg.) and of mothers receiving the milk ration fortified with copper, iron and manganese (average 0.0026 mg.) differed only slightly when estimated on the basis of the amount per animal (table 2). Estimations of the manganese content per kilogram of moist weight were somewhat higher for the milk-manganese group than for the stock group; 0.513 mg. per kilogram for the former, and 0.363 mg. per kilogram for the latter. The manganese content of the young

TABLE 2

Relation of the diet of the mother to the manganese content of the young at birth

RAT NO.	DIET	AGE OF MOTHER AT BIRTH OF YOUNG	LITTER SEQUENCE	NUMBER OF YOUNG BORN	WEIGHT AT BIRTH	NUMBER OF YOUNG ANALYZED	MANGANESE Per Animal	MANGANESE Per kilogram moist weight
		Weeks			*gm.*		*mg.*	*mg.*
60	Milk-Cu-Fe-Mn	13.0	1	9	4.9)	14	0.0028	0.526
64	Milk-Cu-Fe-Mn	13.0	1	5	6.0)			
65	Milk-Cu-Fe-Mn	13.5	1	6	5.2)	15	0.0024	0.502
66	Milk-Cu-Fe-Mn	13.5	1	9	4.8)			
11	Stock food-Mn	12.0	1	6	6.5)	19	0.0019	0.328
12	Stock food-Mn	14.0	1	13	5.5)			
18	Stock food-Mn	14.0	1	7	5.7)	14	0.0024	0.407
19	Stock food-Mn	14.0	1	7	5.6)			
8	Stock food-Mn	...	3	11	6.2	11	0.0023	0.370
1 [1]	Milk-Cu-Fe	14.0	1	7	4.8)			
2 [1]	Milk-Cu-Fe	17.0	1	9	5.1)	21	0.0007	0.147
3 [1]	Milk-Cu-Fe	13.0	1	7	3.8)			
5 [2]	Milk-Cu-Fe	16.0	1	8	5.1)	13	0.0006	0.124
7 [2]	Milk-Cu-Fe	18.0	1	5	6.8)			
1 [1]	Milk-Cu-Fe	20.0	2	7	5.3)	15	0.0006	0.107
2 [1]	Milk-Cu-Fe	22.0	2	8	4.9)			

[1] Placed on diet of milk-Cu-Fe at 8 weeks of age.

[2] Placed on diet of milk-Cu-Fe at 11 days of age.

of rats receiving the same milk ration without the manganese, estimated on moist weight, was 75 per cent lower than those of the milk-manganese group and 65 per cent lower than the stock group. Young of mothers receiving the manganese-deficient diet at 11 days contained only slightly less than those which were placed on the diet somewhat later. Second litters of the manganese-deficient mothers also contained slightly less than first litters.

An estimation of the daily ingestion of manganese by the three groups during gestation shows a parallelism between ingestion and storage during fetal life. Animals receiving the milk, copper and iron ingested an average of approximately 0.022 mg. of manganese per day, whereas those receiving the same diet with added manganese and the stock diets obtained 1.202 mg. and 0.816 mg. per day, respectively.

The causes of the loss or gain in weight during lactation are little understood. Sure has related loss in weight of mothers receiving milk diets to the low vitamin B of the milk. In the milk-fed animals herein reported the weight gain or loss of the mothers on the various diets during lactation did not differ materially. Omitting the animal which lost 16 per cent during an alimentary tract infection manifested by diarrhoea, the mothers receiving the low-manganese diet lost slightly less than any other group studied including the stock animals (table 1). These data, therefore, seem to add further evidence that milk does not supply too little vitamin B for the lactating mother and the needs of six sucklings. The amount of milk taken by the animals during lactation on the two types of milk modification did not differ materially (table 3). Manganese, therefore, would seem to bear no relation to appetite, again indicating that the infant mortality of milk-fed rats is not due to a vitamin B deficiency.

The manganese content of the young of the stock animals at birth averaged 0.002 mg. per animal, or 0.363 mg. per kilogram of moist weight. Shortly after weaning (33 to 36 days) while the amount for a given animal had increased to 0.013 mg., the amount estimated on the basis of weight (average 0.153 mg. per kilogram) had decreased considerably (table 4). From weaning on, the animals receiving the milk-copper-iron ration continued to store small amounts of manganese, the amount being directly proportional to their increase in weight so that estimated on the basis of weight

TABLE 3

Relation of manganese to milk ingestion during lactation

RAT NO.	LITTER SEQUENCE	LACTATION PERIOD: 21 DAYS			YOUNG RAISED	
		Weight of mother average	Weight gain or loss	Milk consumed per day	Number	Weight at 21 days
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol. + 1.5 mg. Mn per 100 cc.						
Third generation						
		gm.	*Per cent*	*cc.*		*gm.*
36	1	164	— 5.7	89	6	22.2
41	1	190	— 5.7	105	5	35.2
41	2	226	— 6.5	99	6	33.3
37	1	194	— 2.5	125	6	34.1
37	2	240	— 3.7	130	6	44.5
79	1	170	— 4.1	107	6	31.8
79	2	238	+ 0.8	111	5	32.0
Fourth generation						
44	1	220	— 4.1	126	6	44.3
45	1	206	— 3.1	115	6	41.5
43	1	225	+ 1.0	162	6	45.8
42	1	207	— 8.0	112	6	34.6
40	1	207	— 5.6	126	6	40.0
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol.						
1	2	218	+ 0.6	152	6[1]	43.3
1	3	228	+ 4.7	136	6[1]	41.6
2	1	193	— 0.0	60	4[1]	33.0
2	2	213	— 0.8	77	6[1]	28.0
2	3	218	— 0.9	77	6[1]	31.3
5	1	190	+ 1.6	107	6[1]	38.0
5	2	203	—13.0[2]	87	6[1]	31.3
6	3	218	— 2.7	97	6[1]	34.8
7	1	204	+ 0.5	108	6[1]	38.3
7	2	232	+ 0.8	152	6[1]	48.3
8	1	195	— 0.0	88	6[1]	30.0

[1] Young transferred from stock.

[2] Diarrhoea during excessively hot weather.

the amount contained in a given animal was approximately the same at 33 to 36 days and at 61 to 63 days, namely, 0.139 mg. and 0.142 mg. per kilogram, respectively.

With the animals which received the milk-manganese ration, the results were quite different. In the first 2 weeks after weaning, there was a rapid storage, five-fold and over when estimated on the basis of the individual animal, and three-fold estimated on the basis of moist weight. After this initial rapid storage, the amount remained constant at approximately 0.080 mg. per animal, or 0.40 mg. per kilogram till the seventy-eighth day, the beginning of the gestation period.

TABLE 4

The relation of diet to the manganese content of the young at various ages

DIET	AGE	NUMBER OF ANIMALS	WEIGHT AVERAGE	MANGANESE PER ANIMAL		MANGANESE PER KILOGRAM	
				Average	Range	Average	Range
	Days		*gm.*	*mg.*	*mg.*	*mg.*	*mg.*
Stock	Birth	44	5.8	0.002	0.002–0.002[1]	0.363	0.328–0.406
Milk-Cu-Fe	33–36	3	88.1	0.013	0.013–0.014	0.153	0.146–0.165
Milk-Cu-Fe	47–50	3	127.6	0.018	0.017–0.019	0.139	0.121–0.161
Milk-Cu-Fe	61–64	3	144.6	0.020	0.018–0.022	0.143	0.110–0.162
Milk-Mn-Cu-Fe	47–50	3	139.5	0.079	0.072–0.082	0.573	0.475–0.686
Milk-Mn-Cu-Fe	61–64	3	192.3	0.083	0.080–0.086	0.404	0.391–0.503
Milk-Mn-Cu-Fe	73–75	3	220.0	0.088	0.087–0.088	0.399	0.389–0.407

[1] The manganese content of the young of stock animals, analyzed in groups of 19, 14 and 11, averaged 0.0019, 0.0023 and 0.0022 mg. per animal, respectively.

COMMENT

The results of the investigation point to the conclusion that the high mortality of the young of animals receiving the milk-copper-iron diet is due to congenital debility directly related to a deficiency of manganese in the diet of the mother during gestation. The young, although of normal size at birth, are not sufficiently robust to survive the ordeal of birth; the few which do survive are not strong enough to suckle. It would seem, therefore, that manganese is an essential constituent of the developing organism. If the diet of the mother during gestation is adequate, the young are born with a store of manganese which is surprisingly constant. The amount per animal at birth of our stock groups (average 0.002 mg.) as well as those receiving the milk-manganese diets (average 0.003 mg.) was found to be within the limits of error of that reported by Skinner, Peterson and Steenbock ('31) in the young of rats receiving their stock diet with the manganese addition.

During suckling there is considerable increase in the manganese store, suggesting that the milk of the rat is somewhat higher than that of the cow, since rats receiving cow's milk without manganese subsequent to weaning store little. Following weaning, storage of manganese takes place rapidly if sufficient is contained in the diet. After 2 weeks on the milk supplemented wtih 1.5 mg. of manganese per 100 cc., the amount of manganese per animal (average 0.079 mg.) was found to be only slightless less than that found 4 weeks later (average 0.088 mg.). Following the initial increase, the amount stored, estimated on body weight, had decreased 30 per cent in the 4 weeks of the study. This percentage decrease suggests that storage was confined to certain tissues rather than to the body as a whole, the tissues most avid for manganese being, according to Skinner, Peterson and Steenbock, bone, brain, hide, kidney and liver.

Although failure in lactation may occur in animals raised on diets quite free from manganese, as suggested by Orent and McCollum, adequate lactation is possible on a diet which contains too little manganese for the development of normal young in utero. Cow's milk containing 0.03 mg. per liter (the Guernsey milk used) supplies sufficient manganese for lactation, since animals receiving this low-manganese diet from infancy were able to suckle successfully six vigorous foster young. In one case three successive foster litters were raised by a rat receiving milk modified with only iron and copper.

The addition of manganese to the milk diet was without influence on the milk consumed by the rats during lactation. This was reflected in the weaning weights of the young and the consistency of the weights of the mother receiving the milk ration during lactation.

SUMMARY

In an endeavor to determine the cause of the high infant mortality of milk-fed rats, reproduction, lactation and the manganese content of the young at birth, at weaning, and thereafter at various periods to sexual maturity have been compared in animals receiving Guernsey milk plus copper and iron, with those receiving the same ration fortified with manganese, as well as with stock fed animals. The lactating ability of the animals receiving the simple milk ration was specifically tested by exchanging their young for vigorous stock young. In a number of instances where the young of the milk-manganese low animals died, they also were given stock young to raise. Records were kept of the amount of milk consumed by each animal during lactation.

The results of the investigation point to the following conclusions:

1. The high percentage of deaths of the young of animals reared on milk diets modified with copper and iron is due to congenital debility, the result of too little manganese in the diet of the mother. Young born of these milk-fed animals were found to contain 65 per cent less manganese than the young of animals receiving a similar ration with added manganese.

2. Animals reared from weaning on a milk diet with no added manganese are able to suckle vigorous normal young to average weaning weights comparable to those of stock young or of young receiving milk with manganese added.

3. Storage of manganese in the young during the suckling period is greater than subsequently on a cow's milk ration, but less than during a subsequent period on a milk ration with 1.5 mg. of manganese per 100 cc. of milk, suggesting that rat's milk is higher in manganese than the Guernsey milk tested.

4. The milk ingestion of rats during the lactation period is not influenced by the amount of manganese the milk contains. Rats receiving the simple milk ration consumed as much milk as those receiving the milk-manganese ration.

5. The Guernsey milk tested supplied enough vitamin B for the lactating mother and the growth needs of six young.

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RESPIRATORY METABOLISM STUDIES IN HYPERINSULINISM FOLLOWING INGESTION OF GLUCOSE[1]

I. M. RABINOWITCH AND A. F. FOWLER
WITH THE TECHNICAL ASSISTANCE OF M. MOUNTFORD AND P. HOLROYDE
Department of Metabolism, The Montreal General Hospital, Montreal, Canada

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Hyperinsulinism is now a well-recognized clinical entity. There is, however, as yet, much to be known with regard to the conditions which lead to, and which may result from, this disturbance. It was, therefore, considered of interest to investigate the respiratory metabolism, in order to determine whether such studies might reveal some as yet unrecognized metabolic defect. As will presently be shown, respiratory metabolism studies are also of value otherwise. They may be of diagnostic value since, in the interpretation of blood sugar time curves in suspected cases, they help to differentiate between apparent and real failure of absorption of glucose from the gastro-intestinal tract. The literature of hyperinsulinism has been repeatedly reviewed and, for purposes of brevity, reference will be made to relevant facts only.

Though hypoglycemia was produced experimentally many years ago and has been known to exist in a variety of clinical disorders, no signs nor symptoms, nor disturbances noted experimentally, were ever attributed to lowering of blood sugar until the discovery of insulin. Experiences with insulin shock in diabetes led to the recognition of a symptom complex identical with insulin shock, but independent of insulin injections. Harris ('24) first drew attention to this condition to which he gave the name hyperinsulinism.

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As the fundamental disturbance is reduction of blood sugar, it is obvious that the term hyperinsulinism may at times be a misnomer, for hypoglycemia may be produced in a variety of ways other than by excess production of insulin. Hypofunction of the thyroid, adrenals and anterior pituitary bodies are examples. As the liver is also intimately concerned with the regulation of blood sugar, a variety of disorders of this organ may also lead to hypoglycemia. As is well known, hypoglycemia may be produced in the experimental animal by hepatectomy and administration of a variety of liver poisons (phosphorus, chloroform, etc.). Clinically, a variety of conditions cause destruction of liver tissue and thus tend to simulate, metabolically, experimental hepatectomy (carcinoma, acute yellow atrophy, etc.). Wauchope ('33), in an excellent review, discussed the morbid anatomy of hypoglycemia and classified symptoms according to organs affected. It would thus appear that, for purposes of exact terminology, hyperinsulinism should be confined to conditions in which there is definite proof of production and liberation of insulin in excess of the normal. The case reported by Wilder et al. ('27) may be cited as an example of true hyperinsulinism; operation and autopsy showed a carcinoma of the pancreas which originated from the islet cells. A large yield of insulin was obtained from the metastases in the liver. The term, hyperinsulinism, might thus also be applied when symptoms are associated with adenomata of the islet cells of the pancreas. Surgical removal of such adenomata has resulted in cure of the symptoms. All other cases of hypoglycemia in which no definite proof of cause can be found might be classified as spontaneous, or idiopathic, hypoglycemia. Such classification is, however, neither simple nor practical. As has been pointed out, the case reported by Wilder et al. still stands alone in completeness of proof of hyperinsulinism and, as Harris ('34) has recently observed, if this criterion is necessary, few diagnoses will be made before operation or autopsy. The important fact, clinically, is recognition of the condition. Whether the disturbance is due actually to excess production of insulin or abnormal activity of the normal supply because of decreased activity of the thyroid, pituitary, adrenal, or other anti-insulin secretion, is largely of academic interest. Clinically, the signs and symptoms are essentially the same and treatment is the same. Regardless of cause, diet alone suffices; in mild cases, frequent feedings of liberal quantities of carbohydrates usually suffice; in more severe disturbances, high fat diets are, at times, of value, as they tend to cause resistance to insulin. Thyroid medication is of value, and intravenous injection of glucose may be necessary in the more severe cases. When these fail, surgical treatment must be considered (removal of tumor, partial pancreatectomy, etc.). As yet, treatment with anterior pituitary gland is of no value. In rare cases, as with insulin injections, the condition, unless promptly relieved, may be fatal. For this reason, and as there are no means of predicting results, severe disturbances should be treated by prompt use of glucose intravenously.

Classification of hypoglycemia on the basis of etiology is, as yet, not very satisfactory for other reasons. Too little is known, clinically, to attribute hyperinsulinism to the thyroid alone; to the adrenals alone or to other organs of internal secretion. There is, as yet, much to be known about the regulation of blood sugar. The dysinsulinism due to rhythmic activity of the liver with respect to storage and liberation of glycogen is an example of the many difficulties in the interpretation of data (Mollerstrom, '33). Whether this rhythmic activity is controlled by some mechanism in the liver or whether it is due to some extra-hepatic activity, such as alternate stimulation and depression of the insulin-producing mechanism, is, as yet, not known. Classification, according to symptomatology, is further complicated by the finding of symptoms without hypoglycemia; symptoms have been reported when the blood sugar was normal or even above normal.[2] The latter finding does not fit in with a recent statement by Harris ('33) that hyperinsulinism is invariably associated with hypoglycemia. Our experiences agree with those of Harris. Among diabetics we have not as yet observed insulin reactions without hypoglycemia. Classification is further complicated by the fact that there may be hyperinsulinism without symptoms. In a case reported by Peters and Rabinowitch ('29) there were for a time no symptoms whatever, though the glucose had disappeared from the blood entirely.

As with insulin injections in diabetes, there are a number of conditions which may influence the severity of symptoms. Insulin, as is well known, acts best in young people, in the undernourished, and in the dehydrated individual. Exercise aggravates the symptoms of excess insulin dosage and the disturbance is likely to be severe whenever there is interference with the absorption of food (gastritis, enteritis, etc.). Symptoms tend to be severe in all conditions which tend toward starvation because of depletion of glycogen (diarrhea, vomiting, etc.). Response to insulin is also increased by low fat diets and symptoms may be severe when there is sudden improvement of carbohydrate tolerance. Sudden improvement is common in recovery from infection, injury and a variety of other conditions which tend to cause resistance to injected insulin or to that normally produced in the body (Rabinowitch and Bazin, '24, '28 and '32). Recently, Harris ('33), in a review of the literature, again drew attention to another disturbance of the insulin-producing mechanism, namely, dysinsulinism, a condition in which hyperglycemia may alternate with, or be followed by, hypoglycemia.

Aside from the importance of early diagnosis in severe cases, recognition of hyperinsulinism is necessary because of other possible sequelae. Hyperinsulinism is a nutritional disorder. Harris, for example, suggests that prolonged over-function of the islet cells of the pancreas may lead to their exhaustion and thus to diabetes. Hyperinsulinism also tends to cause obesity, another important etiological factor in diabetes. It is of interest here to note that hypoglycemia is, apparently, as common as hyperglycemia (Sippe and Bostock, '33).

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[2] Under these conditions, the symptoms might be due to a rapidly falling blood sugar alone or possibly to a decrease of sugar in the central nervous system below the critical level without a corresponding decrease in the peripheral blood (Harrison, '26).

METHODS OF INVESTIGATION

Selection of cases. Though not proved anatomically, the subjects investigated were, judging from clinical signs and blood sugar data, suffering from hyperinsulinism. The history in each case was essentially the same, namely, attacks of hunger and weakness which came on after meals, varying between ½ and 3 hours, in spite of what appeared to be the usual and, at times, more than the usual amount of food. In one case, headache accompanied the weakness and hunger. In all cases, the symptoms were completely relieved by taking food. Two of the subjects discovered that eating between meals relieved the symptoms.

EXPERIMENTAL

The experimental procedures were the same as those which have been in use in this laboratory since 1924 (Finley and Rabinowitch, '24). Experiences with hundreds of such tests have increased our confidence in this type of work. They consist of simultaneous determination of blood sugar and respiratory metabolism before and, again, 30, 60, 120 and 150 minutes after administration of glucose. The vagaries of this experimental procedure are many. A variety of precautions are necessary in order to be reasonably certain that the data obtained reflect the metabolic conditions. It may here be observed, it does not necessarily follow that the respiratory quotient at the mouth is the respiratory quotient in the body tissues. There are, first, the many sources of error due to technic. Adjustment of gas masks or mouth pieces; leakage of gas from the bags in which the samples are collected, and errors during gas analyses are the more common sources.

Carefully trained technicians, care of apparatus and long experience with such tests tend to reduce these errors to a minimum. All gas analyses in this investigation were made with the Haldane type of gas apparatus. Accuracy of apparatus was repeatedly checked by analyses of outside air. All analyses were made in duplicate by two technicians. Each gas sample was, therefore, analyzed in quadruplicate. Urinary nitrogen estimations were not made, because of the uncertainty that values obtained at short intervals of time represent the protein metabolism for the corresponding periods. As these subjects were not diabetics—as there was no glycosuria—15 per cent of the total heat production was attributed to protein. For the validity of this procedure, there appear to be sufficient data (Voit, '01). The remaining calories were assumed to be derived from carbohydrate and fat and were apportioned according to the Zuntz-Schumburg tables as modified by Williams, Riche and Lusk ('12). In the calculations of rates of oxidation of carbohydrate, no alterations were made when the respiratory quotients were greater than 1.0 or less than 0.7. In the former case, consumption of oxygen and carbon dioxide production were attributed to oxidation of carbohydrates and, in the latter to oxidation of fat.

Aside from the sources of error due to technic, a variety of other factors must be considered in the interpretation of data. Psychic influences, for example, must be reduced to a minimum. This depends largely upon the personality and training of the technician. Careful attention throughout the test to respiratory movements, to rate and amplitude of respirations, is very important. The influences of the latter will be dealt with in a discussion of the data.

All tests were performed in the morning and under strictly basal metabolism conditions with no alterations whatever in the dietary habits of the individual prior to the day of the test.

In spite of all of the above-mentioned precautions, there remain the possible sources of error inherent in short time experiments. Though the data obtained under these conditions may actually reflect the metabolism at the time of the test, they may not be representative of the conditions during the greater part of the day. Consideration must also be given to a variety of other factors. As these have been dealt with elsewhere (Joslin, '24; Du Bois, '24), they will not be discussed here. Confidence in this procedure is, however, enhanced by the fact that data of some of our previous experiments have been repeatedly confirmed with the aid of the very exact respiration calorimeter at the Russell Sage Institute of Pathology. The latter include the observations of Ladd and Richardson on renal glycosuria ('25) and those of McClellan et al. ('28) on the metabolism of dihydroxyacetone.

The data of five cases are reported in the order in which they were obtained (tables 2 to 6). In all, six cases were studied. One case is not included in this report, because the blood sugar time curve obtained during the test was not flat; the hyperglycemic response to the ingested glucose was normal. In this investigation, for a nmber of reasons to be dealt with later, we were concerned particularly with flat, or nearly flat, curves.

STANDARDS

Blood sugar time curve. The normal blood sugar time curve is too well known to require comment. The conditions necessary to obtain reliable data were dealt with elsewhere by one of the writers (Rabinowitch, '34).

Respiratory exchange. The average respiratory quotient during the fasting period is 0.82 {0.83 for males and 0.81 for females} (Benedict et al., '14). After the administration of 100 gm. of glucose, an increase may be noted within a few minutes (Higgins, '16) and rarely fails to occur within 30 minutes. The average maximum increase is about 0.12. In some instances a slight decrease may be noted. In conditions which deplete glycogen, such as exercise, starvation, etc., no increase or an actual decrease may be noted for a time. Benedict and Carpenter ('18) found a fairly consistent picture in the ten cases observed. The lowest, highest, and average peaks of carbon dioxide production were, respectively, 12, 35, and 25 per cent above the basal period; of oxygen consumption, 3, 22, and 12 per cent; of the heat production, 6, 24, and 14 per cent; and of the respiratory quotients, 0.07, 0.17 and 0.12, respectively. The average time of appearance of the maximum effect was within 2 hours.

DISCUSSION OF RESULTS

The blood sugar time curves obtained in the five cases in which respiratory metabolism studies were made are shown in table 1. It will be observed that the results, in each case, were essentially the same when the tests were repeated. The time interval between tests ranged from 4 days (subject MS) to about 8 weeks (subject 98–34). In one case (subject 295–34) the test was performed three times with a time interval of 8 days between the first and second test and 7 days between the second and third test. These curves, incidentally, demonstrate the value of the test in the detection of hyperinsulinism. As Wilder ('33) has observed, blood sugar time curves obtained singly may not be very helpful. Repeated tests, however, are helpful, when both curves are flat. The value of

TABLE 1

Showing repeated blood sugar time curves in hyperinsulinism

	7727/33		98/34		1000/34		M. S.		295/34		
	Feb. 16	Feb. 21	Jan. 5	Feb. 28	Feb. 22	Mar. 2	Mar. 15	Mar. 19	Jan. 30	Feb. 7	Feb. 14
Before glucose	0.085	0.093	0.088	0.090	0.100	0.098	0.102	0.104	0.108	0.087	0.116
30 minutes later	0.129	0.112	0.120	0.096	0.122	0.131	0.100	0.123	0.126	0.104	0.128
60 minutes later	0.119	0.137	0.102	0.098	0.129	0.128	0.100	0.123	0.125	0.102	0.135
120 minutes later	0.087	0.097	0.095	0.098	0.091	0.100	0.082	0.089	0.122	0.100	0.125
150 minutes later	0.092	0.087	0.083	0.095	0.080	0.100	0.098	0.083	0.111	0.092	0.117

repeated tests is, however, destroyed when any changes are made in the dietary habits of the individual between tests (Rabinowitch, '34). The following case of hyperinsulinism (subject JBM) is an example of the disturbing effects of diet. In this case, three blood sugar time curves were obtained with the following results:

	Blood sugar (per cent)		
	Feb. 14	Feb. 26	Mar. 14
Fasting	0.111	0.082	0.112
30 minutes after glucose ingestion	0.116	0.100	0.166
60 minutes after glucose ingestion	0.139	0.122	0.149
120 minutes after glucose ingestion	0.105	0.091	0.128
150 minutes after glucose ingestion	0.103	0.075	0.106

It will be observed that the time interval between the first and second tests was 12 days and between the second and third tests it was 16 days. The first two curves are practically flat; whereas, in the third curve (March 14) the hyperglycemic response to the ingested glucose is normal. Between the second and third tests, the diet was increased not only with respect to carbohydrates, but, also, to caloric content.

In spite of all precautions, flat curves, even when obtained repeatedly, need not necessarily be due to hyperinsulinism; they may still be due to some unrecognizable defect in the absorption of glucose from the alimentary tract. As will presently be shown, however, interpretation of blood sugar data may be simplified by simultaneous study of the effects of the ingested glucose upon the respiratory metabolism. McClelland and Wardlaw ('32) first reported respiratory metabolism studies in hyperinsulinism. As far as could be ascertained from the literature, however, the following present study is the first in which blood sugar and respiratory metabolism data were obtained simultaneously.

Case 1. It will be observed (table 2) that the blood sugar time curve was flat; there was no hyperglycemic response to the ingested glucose. This could have been due to defective absorption of the glucose from the gastro-intestinal tract or to rapid disappearance of the absorbed glucose from the blood

TABLE 2

Hospital no. 98/34. Female. Age 21 years. Height 168.5 cm. Weight = 60 kg. Body surface = 1.67 sq.M. B.M.R. = minus 14 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED[1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	8.90	...	10.90	...	0.297	0.817	0.818	52.04	...	4.309	..	12	..	17.13	27.02	7.89	4.28	2.97	1.92	0.090
30 minutes after glucose	9.08	2.0	11.61	6.4	0.313	0.782	0.777	54.93	5.6	3.987	..	11	..	11.75	34.87	8.32	2.94	3.83	2.03	0.096
60 minutes after glucose	10.09	13.4	12.50	14.7	0.340	0.806	0.807	59.51	14.4	4.410	2.3	11	..	17.87	32.61	9.02	4.47	3.58	2.20	0.098
120 minutes after glucose	9.63	8.2	11.36	4.2	0.312	0.848	0.856	54.70	5.1	4.308	..	11	..	24.32	22.09	8.29	6.08	2.43	2.02	0.098
150 minutes after glucose	9.35	5.1	10.94	0.4	0.301	0.855	0.864	52.63	1.1	4.298	..	11	..	23.40	21.26	7.98	5.85	2.34	1.95	0.095

[1] Assumed to account for 15 per cent of total heat production.

stream, either because of rapid oxidation or storage. It will be noted that the respiratory quotient at the 30-minute period was depressed. This, according to our previous experiences with such tests (Rabinowitch, '25), suggested storage of the ingested glucose. The increase of heat production which was quite appreciable at the 60-minute period is, however, difficult to interpret. It was apparently not due to carbohydrate oxidation and can hardly have been due to stimulation of peristalsis by the ingested fluid. It is a well-known fact that the effects of cathartics and agar-agar are almost negligible, in spite of the intestinal activity which they produce. Without an increased elimination of carbon dioxide (washing out phenomenon), it is difficult to attribute the increase of heat production to excess activity of the respiratory muscles. Oxidation of food stuffs, other than carbohydrate, is the most likely explanation of the increase of heat production.

The respiratory quotient at the end of 120 minutes was increased and, because of the precautions taken, this finding is not regarded as an artefact due to washing out of carbon dioxide. It will be noted that the ventilation and respiration rates also fail to suggest over-ventilation; they were remarkably uniform throughout the test and practically identical with conditions in the fasting (control) period. The cooperation of the patient was ideal. The heat production at this period was increased, but the increase was lower than that which corresponds to the usual specific dynamic action of glucose. It is, however, difficult to attribute it to some other condition. There was no obvious excess activity of the muscles of respiration, nor were there any other abnormal movements. The time factor tends to exclude effects of water ingestion.

In view of the inappreciable increase of heat production at the 150-minute period, the increase of the respiratory quotient is attributed to storage of the ingested glucose, possibly by conversion into fat. Here, also, the ventilation and respiration rate data appear to exclude an artefact due to washing out of carbon dioxide. On the whole, therefore, it would appear that, in this case of suspected hyperinsulinism, the general response to the ingested glucose was storage. Since we believe that all recognizable technical and other errors have been excluded, this case affords an example of the value of respiratory metabolism studies when the blood sugar time curve is flat. In this case, defective absorption of the ingested glucose from the alimentary tract is excluded. That our interpretation of the above data is correct is suggested from the experiences with the following case.

Case 2. In this case (table 3) the blood sugar time curve was practically flat; but, differing from the previous case, it will be noted that, following ingestion of the glucose, there was an immediate and sustained increase of the respiratory quotient; and each increase was accompanied by an increase of heat production. Because of the ventilation and respiration rate data, we believe that artefacts due to washing out of carbon dioxide are excluded. It is of interest to note that the percentage increase of carbon dioxide elimination was appreciably greater than the percentage increase of ventilation rate; and that the latter bore little or no relationship to the percentage increase of heat production. In this case of suspected hyperinsulinism, therefore, the dominant response to the ingested glucose was oxidation.

Proof that the flat blood sugar time curve in this case was not due to defective absorption of glucose is much more definite than in the previous case.

Assuming these two individuals were suffering from hyperinsulinism, it would appear that the response to glucose ingestion in this condition is not uniform. Further suggestive are the findings when consideration is given to the dietary habits of these subjects; the data fit in with the generally accepted views of carbohydrate oxidation in animals and with findings in man. In simultaneously determined arterial and venous blood sugar and respiratory quotient time curves in diabetics following insulin administration, Rabinowitch and Bazin ('27) showed that, as in animals, glycogen formation appears to be a necessary preliminary to oxidation of carbohydrates. This offers an explanation of the data in the above-

TABLE 3

Subject M.S. Female Age 26 years. Height 167.6 cm. Weight = 60.4 kg. Body surface = 1.67 sq.M. B.M.R. = minus 3 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED[1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	10.02	...	12.14	..	0.331	0.825	0.829	58.04	...	4.98	...	12	..	20.78	28.47	8.79	5.20	3.13	2.14	0.104
30 minutes after glucose	11.10	10.8	13.08	7.7	0.359	0.849	0.856	62.97	8.5	4.96	...	11	..	28.00	25.43	9.54	7.00	2.79	2.33	0.123
60 minutes after glucose	11.34	13.2	12.68	4.4	0.352	0.895	0.911	61.66	6.2	5.63	13.0	11	..	36.31	16.01	9.34	9.08	1.76	2.28	0.123
120 minutes after glucose	11.99	19.7	12.64	4.1	0.356	0.949	0.977	62.38	7.5	5.44	9.2	10	..	49.33	3.60	9.45	12.33	0.39	2.30	0.089
150 minutes after glucose	11.42	14.0	12.47	2.7	0.349	0.916	0.937	61.02	5.1	5.04	1.2	10	..	41.21	10.56	9.25	10.30	1.16	2.26	0.083

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[1] Assumed to account for 15 per cent of total heat production.

mentioned cases of hyperinsulinism. It would thus appear that the response to glucose in hyperinsulinism depends upon the amount of glycogen stored in the body at the time of the test. With a good supply of glycogen, the response to glucose ingestion is oxidation and when there is a tendency toward depletion of glycogen, the initial reaction is storage. The findings in these two cases fit in with the respiratory metabolism data obtained by McClellan and Wardlaw ('32) in another case of hyperinsulinism. They also fit in with the following cases in which the dietary habits of the subject were carefully correlated with the metabolic data. The importance of a knowledge of the dietary habits, in the interpretation of respiratory metabolism data, was emphasized by one of the writers in 1925 in somewhat similar studies (Rabinowitch, '25). In the above-mentioned paper, McClellan and Wardlaw again draw attention to this fact.

Case 3. There are a number of difficulties in the interpretation of the data in this case. Here, also, it will be noted (table 4) that, as in case 2, the blood sugar time curve was practically flat and that the respiratory quotients were increased throughout the test. There are, however, two important differences between the two cases; the respiratory quotient was very low in the fasting state (lower than the respiratory quotient for fat) and it was very high (higher than the respiratory quotient for carbohydrate) at the 120-minute period.

Very low respiratory quotients, less than 0.7, are not very common. They may be met with in the very severe diabetic when the D:N ratio is very high. The late Prof. Graham Lusk ('28) calculated that the diabetic respiratory quotient for protein is 0.632 when the D:N ratio is 3.65. Respiratory quotients lower than 0.7 have also been noted occasionally in normal individuals. Lyon, Dunlop and Stewart ('32) have recently reviewed the literature and drew attention to the low values which may be met with in obesity. These authors suggested the possibility of conversion of fat into sugar. Space does not permit a discussion of this highly controversial

TABLE 4

Hospital no. 1000/34. Male. Age 29 years. Height 180.3 cm. Weight = 61.2 kg. Body surface = 1.77 sq.M. B.M.R. = plus 9 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED[1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	10.93	. . .	16.36	. .	0.433	0.668	0.642	76.17	. .	5.584	. . .	13	. .	. . . .	64.67	11.50	. . . .	7.11	2.80	0.098
30 minutes after glucose	12.45	13.9	17.29	6.3	0.460	0.720	0.704	80.47	5.6	5.964	6.8	13	. .	. . . .	68.27	12.19	. . . .	7.50	2.97	0.131
60 minutes after glucose	13.06	19.5	15.03	. .	0.416	0.869	0.881	72.61	. .	5.923	6.1	13	. .	36.46	25.13	11.02	9.12	2.76	2.69	0.128
120 minutes after glucose	13.24	21.1	12.77	. .	0.365	1.037	1.083	63.22	. .	6.203	11.1	13	. .	53.55	. . . .	9.67	13.39	. . .	2.36	0.100
150 minutes after glucose	13.01	19.0	13.42	. .	0.380	0.970	1.001	66.51	. .	6.163	10.4	13	. .	56.42	. . . .	10.08	14.11	. . .	2.46	0.100

[1] Assumed to account for 15 per cent of total heat production.

subject. It is, however, an interesting speculation as to whether, in spontaneous hyperinsulinism, when the body is depleted of its carbohydrates, it attempts to obtain sugar from unusual sources. Another explanation, and which perhaps fits in better with established facts of physiology, is that the low respiratory quotient in hyperinsulinism is due to the protein metabolism. It is suggested that conditions in hyperinsulinism may be, at times, somewhat comparable with those in the very severe diabetic with a high D:N ratio; in the diabetic, the low respiratory quotient is due to the fact that the glucose formed from protein is excreted; in spontaneous hyperinsulinism, when there is a tendency toward depletion of glycogen, the glucose so formed is stored.

That the very low respiratory quotient in this case was due to storage of glucose from protein is suggested from the findings after administration of glucose. It will be observed, as stated, that the respiratory quotient was greater than 1.0 at the 120-minute period. An artificially high respiratory quotient due to over-ventilation is, we believe, excluded. There was no obvious over-ventilation throughout the test and it will be noted that there was no relationship between percentage increase of ventilation rate and percentage increase of carbon dioxide elimination.

Further suggestive of storage of the ingested glucose is the fact that, with one exception only (the 30-minute period), increase of respiratory quotient was not accompanied by increase of heat production. (It should also be observed that the respiration rates were remarkably regular and slow.)

The fact that the data show an increased rate of oxidation of carbohydrates (grams carbohydrate oxidized per hour) is not, in our opinion, incompatible with the above interpretation. The values recorded are obviously due to the arbitrary method of calculating rate of oxidation of carbohydrate. It is, of course, possible that glucose may be oxidized without exerting a specific dynamic action. This peculiar phenomenon has been observed before. Coleman and Du Bois ('15), for example, found no appreciable increase of heat production in typhoid fever during the febrile stage of the disease. This, as Lusk suggested ('14), may have been due to the increase of metabolism. These data are, however, hardly comparable with those obtained in the above case of hyperinsulinism, since there was neither fever nor an increase of basal metabolism. Storage of the ingested glucose is the most likely explanation of our data. Storage, as a matter of fact, is probably the explanation of the absence of specific dynamic action of food in fevers. As Lusk pointed out in his discussion of the protein metabolism in typhoid fever, the protein in fever can never be reduced to as low a level as in the normal individual. Therefore, the protein ingested may serve to replace the protein which is being broken down in excess. Under these conditions, it would not increase the heat production. That the interpretation of our data in the above case of hyperinsulinism is correct is suggested from experiences with the following case:

Case 4. The history of this case is of interest. The patient complained of marked weakness on awakening in the morning; of drowsiness in the afternoon; and of a loss of 20 pounds of weight during the last year. Loss of weight fits in with starvation and, therefore, depletion of body glycogen. Assuming that glycogen formation is a necessary preliminary to oxidation, one could, a priori, expect the first effect of glucose ingestion in this case to be storage. One might also expect, as in case 3, a very low respiratory quotient in the fasting state. It will be noted, however, that the respiratory quotient was not only not low, but much higher than normal (0.907) and it is difficult to attribute it to over-ventilation. Firstly, there was no increase of respiration rate; the respirations were 20 per minute. Secondly, since the ventilation rate was 6.20 liters and the respiration rate was 20 per minute, the average exchange of air was only 310 cc. per respiration and, lastly, the basal metabolic rate was low, namely, minus 9 per cent. This is opposed to excess activity of the muscles of respiration. The high respiratory quotient in the fasting state suggests that, in this case of hyperinsulinism, the body, in

TABLE 5

Hospital no. 7727/33. Male. Age 20 years. Height 167.6 cm. Weight = 59.2 kg. Body surface = 1.66 sq.M. B.M.R. = minus 9 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	11.46	...	12.63	...	0.353	0.907	0.927	61.63	...	6.20	...	20	..	39.84	12.44	9.35	9.96	1.37	2.280	0.093
30 minutes after glucose	13.20	15.2	13.68	8.3	0.387	0.965	0.997	67.74	9.9	6.92	11.6	19	..	57.48	....	10.25	14.37	...	2.50	0.112
60 minutes after glucose	13.60	18.7	14.24	12.7	0.402	0.955	0.984	70.26	14.0	6.88	11.0	20	..	55.56	4.05	10.65	13.89	0.44	2.60	0.137
120 minutes after glucose	13.62	18.8	13.95	10.4	0.396	0.977	1.011	69.10	12.1	6.77	9.2	20	..	58.60	....	10.51	14.65	...	2.56	0.097
150 minutes after glucose	12.80	11.7	11.24	...	0.321	1.139	1.207	55.65	...	7.31	19.5	19	..	47.14	....	8.51	11.78	...	2.075	0.087

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[1] Assumed to account for 15 per cent of total heat production.

spite of loss of weight, had a good supply of glycogen, a supply which permitted continuous oxidation of appreciable quantities of carbohydrate even as late as 15 hours after the last meal. That this is the correct explanation is suggested from the history of the case. This patient learned by experiment that the weakness and hunger which came on regularly about 2 hours after meals could be relieved by eating between meals. He has, therefore, followed this practice for some time. This offers an explanation of the findings after administration of the glucose. It will be observed that, differing from the previous case, the prominent response to the ingested glucose was oxidation. Proof of this is that, with one exception, each increase of respiratory quotient was accompanied by an increase of heat production which corresponded to the normal specific dynamic action of glucose. That this increase of heat production was due to the specific dynamic action of glucose and not to over-ventilation is again suggested from correlation of ventilation rate with carbon dioxide elimination and heat production.

There is one finding difficult to interpret in this case. It will be observed that the very high respiratory quotient at the 150-minute period was not accompanied by an increase of heat production. The respiratory quotient, being greater than unity, suggests conversion of glucose to fat. The increase of ventilation rate suggests an artefact and, according to the clinical history, it would appear that storage of ingested glucose was not necessary. On the other hand, there was not only no increase of heat production, but actually a decrease. This suggests storage.

Case 5. In this case (table 6) some of the data are also difficult to interpret. It would appear that there was a combination of storage and oxidation. As in the previous case, the respiratory quotient is high in the fasting state; and, here, also, the data fit in with the history of the case. This man suffered from diarrhea and loss of weight before the test and these were attributed to a chronic pancreatitis. A high carbohydrate-low fat diet was, therefore, substituted for the

TABLE 6

Hospital no. 295/34. Male. Age 43 years. Height 160.2 cm. Weight = 51.36 kg. Body surface = 1.51 sq.M. B.M.R. = plus 7 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED[1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	11.17	...	12.57	..	0.349	0.889	0.904	61.01	...	6.790	...	..	..	34.16	17.59	9.261	8.539	1.933	2.259	0.116
30 minutes after glucose	14.15	26.7	12.58	0.1	0.359	1.124	1.189	62.32	2.1	7.752	14.1	..	..	52.79	....	9.524	13.198	....	2.323	0.128
60 minutes after glucose	14.09	26.1	12.79	1.7	0.365	1.101	1.161	63.33	3.8	7.216	6.3	..	..	53.65	....	9.683	13.41	....	2.362	0.135
120 minutes after glucose	14.86	33.0	13.70	9.0	0.391	1.085	1.141	67.86	11.2	7.665	12.8	..	..	57.48	....	10.37	14.37	....	2.530	0.125
150 minutes after glucose	14.24	27.5	12.99	3.3	0.371	1.096	1.155	64.34	5.4	7.254	6.8	..	..	54.51	....	9.834	13.63	....	2.398	0.117

[1] Assumed to account for 15 per cent of total heat production.

normal diet 11 days before the test and was continued until 3 days before the test when he was given a full hospital diet. He was also given frequent feedings. Since protein must have contributed appreciably to the heat production during the loss of weight, it would appear that the data are somewhat comparable to those which were obtained in typhoid fever by Du Bois when a high carbohydrate was substituted for a high fat diet. It will be noted that all of the respiratory quotients after administration of the glucose were greater than 1.0 and, that except for the 120-minute period, the increase of heat production was below the minimum specific dynamic action of glucose in normal individuals (Benedict and Carpenter, '18). Over-ventilation does not appear to account for these very high respiratory quotients, according to percentage increase of carbon dioxide elimination, ventilation rate and heat production.

The basal metabolic rate was also normal, namely, plus 7 per cent. The above data are, therefore, attributed to a combination of oxidation and storage. It is suggested that, because of loss of weight due to the chronic diarrhea, there was a tendency to store the ingested glucose as fat, in order to restore body weight. This would account for the respiratory quotients greater than unity. As the patient received a liberal carbohydrate diet for some days before the test, this restored the glycogen in the body. (There was no acetone in the urine.) With a good supply of glycogen, some of the glucose ingested was oxidized.

It was considered of interest to examine the respiratory metabolism data for any harmful effects of hyperinsulinism other than those due to hypoglycemic disturbances. As previously stated, Harris suggested that prolonged over-function of the cells of the pancreas may lead to their exhaustion and thus to diabetes. In our cases, at least to date, there is no evidence of such exhaustion. It will be noted that the blood sugar time curves in all of the above cases were flat or nearly so. Flat blood sugar time curves, when not due to defective absorption of sugar from the gastro-intestinal tract, suggest exceptionally good carbohydrate tolerance.

That exposure to excess insulin may eventually disturb sugar tolerance and need not necessarily do so by exhaustion of the islet cells of the pancreas is suggested from our experiences in 1927,[3] with repeated blood sugar time curves obtained in a normal individual following administration of insulin. The following is an example:

In this case (Hosp. no. 5848/27) the patient was suffering from an eczema and the blood sugar time curve suggested a slight disturbance of carbohydrate tolerance. It was considered of interest to test the effects of insulin upon the course of the eczema. The following are the results of six blood sugar time curves obtained during the period of observation:

	Date (blood sugar—per cent)					
	Mar. 7	Oct. 6	Nov. 5	Nov. 12	Nov. 17	Nov. 23
Fasting	0.106	0.093	0.084	0.114	0.120	0.099
30 minutes after ingestion	0.149	0.175	0.222	0.250	0.232	0.166
60 minutes after ingestion	0.196	0.204	0.250	0.385	0.454	0.250
120 minutes after ingestion	0.101	0.111	0.196	0.416	0.357	0.476
150 minutes after ingestion	0.109	0.106	0.075	0.238	0.181	0.250

The first two blood sugar time curves (Mar. 7 and Oct. 6) were obtained before insulin. The third curve (Nov. 5) was obtained 3 weeks after insulin treatment was instituted. It will be noted that there was a marked disturbance of the carbohydrate metabolism; and according to the subsequent curves the disturbance appeared to be progressive. All six curves were obtained under the strict conditions previously described (Rabinowitch, '34). Errors are, therefore, excluded. This patient was kept in the hospital from October 5th to November 24th and no alterations were made in the diet between tests. In spite of repeated physical examinations, other than the exzema, nothing was found to account for the progressive disturbance of carbohydrate tolerance. Unfortunately, the patient refused further treatment. He was discharged from the hospital and has not been heard of since.

That the relationship noted between the insulin treatment and disturbance of carbohydrate tolerance was causal and not accidental is suggested from somewhat similar experiences in other cases. Interpretation of the findings is, however, difficult. Experimental data with respect to the effects of insulin on normal animals are conflicting. Some authors have found that insulin and sugar administered simultaneously caused an increased storage of glycogen in the body; the insulin so administered produced the same physiological effects as in the diabetic, but with more intensity. Others have failed to observe such effects; not only was there no increase of utilization of carbohydrates, but large quantities of insulin were found to suppress glycogen formation almost completely. In some cases, the course of the ingested glucose could not be traced. The whole subject was excellently reviewed recently by Cori ('31). The findings of Chaikoff and Macleod ('27) are of special interest here. When normal rabbits were fed ample carbohydrates, insulin did not increase carbohydrate oxidation and, from the data available at the time, Macleod ('26) concluded that the difference between the normal and diabetic animal is due to the fact that the normal animal can at all times release from the pancreas a sufficient supply of insulin to metabolize or polymerize whatever amounts of carbohydrate may be present in the body. In other words, the endogenous supply of insulin is always at an optimum in the normal animal.

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[3] Unpublished data.

There are very few respiratory metabolism data showing the effects of simultaneous administration of glucose and insulin in normal human individuals. From the data obtained by Rabinowitch and Bazin ('28) it would appear that, unlike in diabetes, insulin does not increase the rate of oxidation of administered carbohydrates. From data then available, animal and human, it was suggested that insulin does not only not enhance oxidation of sugar in the normal individual, but in some as yet unexplained way interferes with the normal mechanism. The data fit in with more recent findings of other workers. Wilder, Smith and Sandiford ('32), in a study of obesity, noted that administration of insulin for periods of from 12 to 30 days was followed by temporary loss of carbohydrate tolerance to such an extent that the blood sugar time curves were typical of diabetes mellitus. Paul, Clark and Gibson ('32) studied the effects of insulin in a non-diabetic who suffered from anorexia. Hyperglycemia and glycosuria developed when the insulin was discontinued. In a study of five other non-diabetics who were given insulin three times a day in relationship to meals, the results were essentially the same and blood sugar time curves in three of these cases indicated diminished sugar tolerance. Data obtained by McJunkin and Roberts ('32) are of interest here. In a study of the effects of excessive insulin on the pancreatic islet cells in young rats, these workers noted that the injected insulin inhibited the proliferative activity of the islet cells, though the rate of growth of the animals otherwise was not impaired. More recent experiences, therefore, also suggest that injected insulin not only does not enhance oxidation of sugar in normal individuals, but interferes with the action of the insulin produced by the body. Does this also apply to the excess insulin to which the body is exposed in hyperinsulinism? The data in table 7 are of interest here. In this table are summarized

TABLE 7

Showing increases of respiratory quotients and percentage increases of oxygen consumption, carbon dioxide production and heat production in normal individuals following administration of glucose with and without insulin and in hyperinsulinism

	RESPIRATORY QUOTIENT			OXYGEN CONSUMPTION			CARBON DIOXIDE PRODUCTION			HEAT PRODUCTION		
	Low	High	Average	Low	High	Average	Low	High	Average	Low	High	Average
Normal[1]	0.07	0.17	0.12	3	22	12	12	35	25	6	24	14
Glucose and insulin administered simultaneously to normal individuals[2]	0.01	0.14	0.06	1	24	9	0	46	13	1	26	10
Hyperinsulinism	0	0.35	0.15	0	14.7	4.8	2	33	16.9	0	14.4	5.9

[1] Benedict and Carpenter—Carnegie Inst. Wash. Pub. no. 261.

[2] Rabinowitch and Bazin—J. Biol. Chem., vol. 80, p. 723, '28.

the findings of the above five cases of spontaneous hyperinsulinism. These are compared with the results obtained by simultaneous administration of glucose and insulin in normal individuals (Rabinowitch and Bazin, '28) and with the data obtained by Benedict and Carpenter with glucose alone (Benedict and Carpenter, '18). It will be observed that the average values in the cases of hyperinsulinism paralleled more closely those obtained with simultaneously administered insulin and glucose in normal individuals than those obtained with glucose alone. It would, therefore, appear that hyperinsulinism may, at times, not only not cause an increase, but, in some as yet unknown manner, may tend to depress oxidation of carbohydrates.

SUMMARY

Simultaneous blood sugar and respiratory metabolism time curves were obtained in five cases of hyperinsulinism after oral administration of glucose.

In each case, the diagnosis of hyperinsulinism was based upon the history and blood sugar findings; the patients complained of weakness, fatigue, hunger, etc., which appeared within a few hours after meals and were relieved by taking carbohydrate-containing foods; and, in each case, repeated blood sugar time curves obtained after administration of glucose were flat; hyperglycemic response to the ingested glucose was inappreciable or absent.

According to the respiratory metabolism data, the response to glucose ingestion may be oxidation or storage; and it would appear that the type of response depends upon the amount of glycogen in the body. With a poor supply of glycogen, the tendency is toward storage and, when the diet is liberal with respect to carbohydrates, the tendency is toward oxidation.

As in all of these cases the clinical histories and symptoms were essentially the same, it would appear that the symptoms of spontaneous hyperinsulinism are largely independent of the glycogen content of the body and are due essentially to disappearance of sugar from the blood stream. Either oxida-

tion or storage may affect such disappearance. The condition thus resembles insulin shock due to insulin injections.

No proof was found that the rate of oxidation of administered carbohydrates is increased in hyperinsulinism. As a matter of fact, the average values suggested a decrease and corresponded somewhat to those obtained by simultaneously administered glucose and insulin in normal (non-diabetic) individuals.

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HUMAN CALORIMETRY

I. A SEMI-AUTOMATIC RESPIRATION CALORIMETER

JOHN R. MURLIN AND ALAN C. BURTON
Department of Vital Economics, University of Rochester, Rochester, N. Y.

NINE FIGURES

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Since the accurate proof that in a given period the energy output of the animal body is approximately equal to the energy produced by the oxidations occurring in the body in that period, direct calorimetry has taken a position of less general interest. If the energy production may be calculated with confidence from a knowledge of the oxygen consumed, of the urinary nitrogen excreted, and of the respiratory quotient making use of a table such as that of Zuntz and Schumburg ('01), then the measurement of the respiratory exchange suffices for many purposes and requires relatively simple apparatus for its determination. There remains, however, a class of problems that demand for their solution the simultaneous measurement of the respiratory exchange and of the heat output of the body. When processes other than the oxidation of fat, protein and carbohydrate are taking place in the body to any considerable amount, energy and gaseous exchanges of unknown magnitude may be involved and our knowledge of the calorific value of a liter of absorbed oxygen is inadequate. Many such processes are known to be of common occurrence, among them being the conversion of carbohydrate to fat, possibly the reverse conversion of fat to carbohydrate; the change from sugar to glycogen and of glycogen to lactic acid, the influence of various hormones on metabolic processes, etc.

Also in studies of the heat regulating mechanism of the body in normal and disturbed conditions, measurement of the heat elimination as well as of the heat production is, of course, essential. The calorimeter to be described was designed for study of the problems in these fields.

The end in view at its construction was to have a respiration calorimeter of working accuracy, comparatively inexpensive in its initial cost, and as automatic in its operation as possible. The ideal was a machine operable by one man alone. In the measurement of the direct heat, almost complete automaticity has been achieved. The measurement of the respiratory exchange occupies about two-thirds of the time of one man. In routine practice two observers are used, the presence of the second being desirable to see that the automatic features are working properly, to take any additional measurements required in any experiment and to ensure, for the peace of mind of the subject, that the calorimeter is never left unattended.

Certain details of the construction are explained by the fact that it was designed for studies of heat production and heat elimination in fevers produced artificially by diathermy and by the high-frequency condensor field. To this end, glass and other non-conducting materials had to be used instead of metal where the latter perhaps might have been preferred if the accuracy of calorimetry were alone to be considered.

GENERAL PLAN OF MEASUREMENT OF GASEOUS EXCHANGE OF HEAT

Figure 1 is a schematic drawing showing the method of measurement of the respiration calorimeter. For the gaseous exchange the closed-circuit system is used. The air in the air-tight chamber in which the subject lies is forced continuously by the pump through the circuit, which includes a Benedict train of absorbers. Water vapor is absorbed by two bottles of sulphuric acid; carbon dioxide by a soda lime bottle followed by one of sulphuric acid. Two such trains are interchangeable in the circuit by the simultaneous throw of two valves so that one may be weighed while the other is absorbing. Oxygen is admitted automatically as it is used by the subject. Its amount is measured as it enters by a gas meter.

The portion of the heat that is lost from the chamber by evaporation of water is calculated from the amount of water collecting in the acid bottles. Of the remaining heat, lost by radiation and convection, the major part is absorbed by cold water flowing through a grid of glass pipes in the chamber. Before entering, the water is precooled and then brought to a constant temperature in a thermostated bath. The difference of temperature of the water entering and leaving the chamber is measured and automatically recorded by electrical thermometers (T_w in the diagram). The average difference of temperature multiplied by the weight of water flowing in any period, also automatically measured by a water meter of special design gives the number of calories of heat entering the water. A small amount of heat is exchanged with the circulating air. For its calculation the rise of temperature of the air is recorded by the electrical thermometers T_A and the rate of flow of air is read from a simple air flow meter.

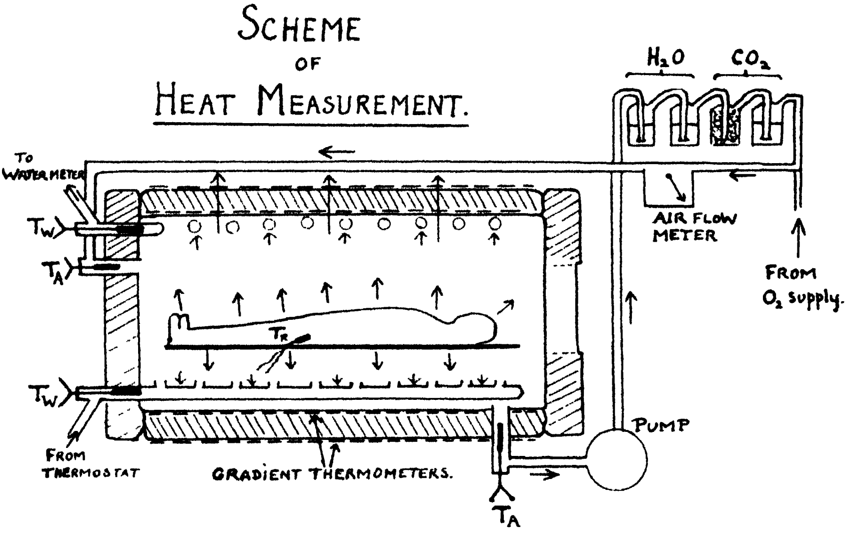

Figure 1

The complicated construction of most of the existing calorimeters arises from the attempt to make them adiabatic. A series of enclosing walls are necessary that may be heated or cooled to prevent the escape or ingress of heat from the chamber except by the absorbing system provided. Great simplification in this calorimeter has been achieved by abandoning the adiabatic principle and instead measuring the heat escaping from the calorimeter into its surroundings. It may be noted that in modern purely physical calorimetry, the adiabatic principle is not in general favor, equal and probably greater accuracy being achieved, after the losses of heat have been minimized, by measurement of the losses that remain. Further, no less accuracy of measurement is required to know that the surrounding walls of the calorimeter are at the same temperature than is needed to measure the small difference of temperature that may exist across the insulation.

In the calorimeter here described, heat loss is reduced by the use of an insulating layer surrounding the calorimeter wall, the remaining heat loss being then measured by the thermal gradient set up by the heat flow across the insulation. Electrical thermometers, one inside, one outside the insulating layer, record the gradient of temperature automatically. Calibration has enabled a graph to be drawn giving the heat loss corresponding to any given gradient.

DETAILS OF CONSTRUCTION

The calorimeter room. The room in which the calorimeter is installed measures 17 feet (5M) by 8 feet 6 inches (2.6M) by 10 feet (3M) high, and was built originally for an incubator room. Its walls and doors are lagged with 3 inches (7.5 cm.) of cork insulation. Four electrical heaters at widely spaced points in the room are regulated by a thermostat hanging above the calorimeter. By introducing a series of pipes carrying brine from the refrigerator plant and balancing the heat output of the electric heaters, a lower range of regulation was secured and the room may now be kept to any temperature between 20°C. and 28°C. with a constancy of about ½°C. The calorimeter chamber, with water circulating system and the air circulating system with the Benedict train, occupy one side of the room. Against the opposite wall are the balance and electrical controls and recording devices (fig. 7).

The chamber. The chamber consists of four cylindrical sections of Pyrex glass, 23 inches (57.5 cm.) in diameter; each section being 19 inches (47.5 cm.) long. The last section is closed at one end. Flanges at the edges of each section enable them to be clamped together with a rubber gasket interposed, by an arrangement of bakelite rings into which bakelite rods are threaded to supply tension and to form a rigid framework round the glass (fig. 2). In addition 'Vulcatex' cement ('knife grade C') is used to seal the joints between the sections. A shorter closed section, 7 inches (17.5 cm.) depth, forms the head of the chamber.[1]

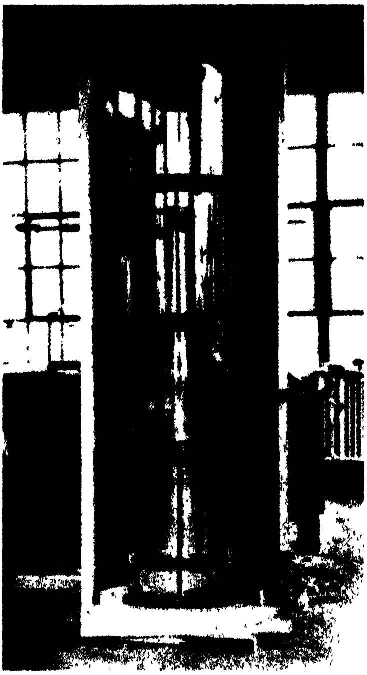

Fig. 2 Glass chamber of calorimeter, showing manner of binding together the segments with bakelite rods and rings.

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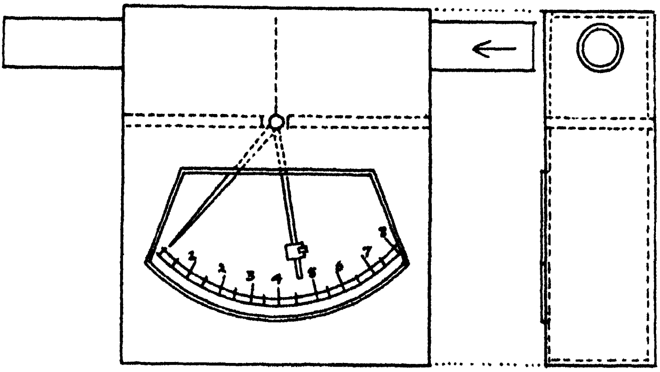
[1] The chamber and mounting were made specially for the calorimeter by the Corning Glass Company, Corning, New York.

The glass chamber is cradled in a hardwood box of external dimensions 29 inches (72.5 cm.) by 29 inches (72.5 cm.) by 82 inches (205 cm.) long. The glass rests upon wooden saddle pieces at each junction of the sections. The last, closed glass section is pressed against the wooden end piece of the box through a rubber pad. A space 1½ inches (3.75 cm.) wide at its narrowest parts exists between the glass cylinder and the wooden walls. It is occupied by the heat insulation to be described later. Several holes for the entrance and exit of the water pipes, the air-flow pipes and of electrical wiring are bored through the glass walls and end piece. A glass window 12 inches (30 cm.) square, in the wooden frame that holds the glass head of the chamber admits light and permits observation of the subject. The enclosing wooden box is supported on two wooden 'horses' so that the axis of the cylindrical chamber is some 2 feet 9 inches (83 cm.) from the floor.

Rapid and effective air-tight closure is made by the arrangement of eight equally spaced steel rods, hinged to the outside of the box so that they swing into engagement with notched metal pieces on the wooden head. Knurled hand-wheels on the threaded rods then clamp the bakelite rings of the head and chamber sections together through a rubber gasket which is cemented permanently to the head. Vaseline is used as a lubricant on the gasket.

The air circulation system. A rotary blower, fitted with a pressure equalizing chamber, is driven at 600 r.p.m. by a 110-volt motor. It is capable of circulating the air through the apparatus at rates up to 60 liters per minute. The rate is controlled by the setting of a tap in a short tube by-passing the air directly from outlet to inlet of the pump. A rate of about 35 liters per minute is usually employed. Brass piping of ½ inch (1.25 cm.) inside diameter leads the air up to the absorbing train on the bench above. The two Benedict trains, each of the four bottles of 2 liters' capacity are mounted in parallel, the air being diverted through one train or the other by the throw of a lever on a rod which turns simultaneously two ground-in metal valves, placed one at each end of the trains. From the trains the air pipe passes under the bench to a bottle containing sticks of sodium hydroxide, to prevent the possible access of acid fumes to the chamber. It then passes through the simple air-flow meter, to be described, to enter the chamber through a hole near the top of the closed end. The air leaves the chamber by an exit hole in the lower surface of the section nearest the open end, and returns to the pump.

Figure 3 shows the simple air flow meter that has been found to be very satisfactory in operation. Brass is used throughout. A dividing shelf makes a 1-inch (2.5 cm.) square channel, which is almost completely blocked by a light vane (in dotted lines in the figure). The vane is pivoted at the level of the base of the channel and carries a pointer and counterpoise beneath so that under the influence of gravity it stands vertically. Flow of air deflects the vane to a new equilibrium position indicated by the pointer on a scale viewed through a glass window. Adjustment of sensitivity is made by movement of the counterpoise weight on its rod. Calibration, made by collection of the air flowing through the meter in a large spirometer, shows that the scale is practically linear in the range of rates of flow used. The rate in liters per minute



AIR FLOW METER

Figure 3

is read from the calibration curve. Owing to the small magnitude of the specific heat and density of air, the item of heat exchange with the air is in any case a small one, and for its calculation this simple meter is quite accurate enough.

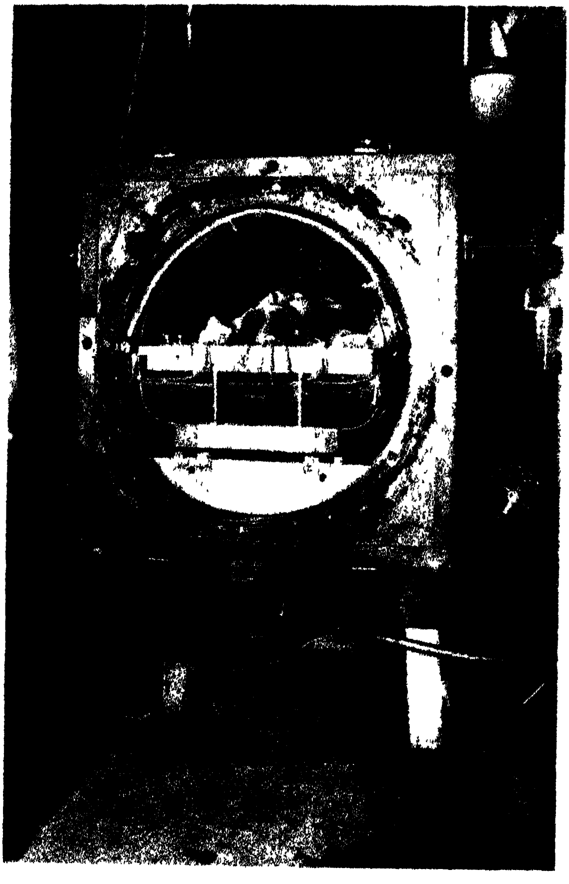


Fig. 4 Chamber open with subject in position. The subject's hand grasps a 'buzzer' button. The arching elements of the glass grid can be seen in the upper part of the chamber.

The oxygen supply. A spirometer of some 4 liters' capacity is connected to the chamber and ensures that the pressure inside is always atmospheric. It may be seen to the right-hand side in the photograph, figure 4. One millimeter movement of the pointer attached to the bell corresponds to a volume change of 18 cc. When the spirometer bell falls below a certain level, a pin on the counterpoise pulley pushes down an arm making contact with mercury in a cup. This contact actuates a relay, operated on 3 volts, which closes a 110-volt current to energize a magnetically controlled 'pinch cock' admitting oxygen from a cylinder into the system. As the spirometer then rises, a spring keeps the arm in contact until the pin eventually lifts it from the cup, thus breaking the circuit and stopping the inflow of oxygen (Riche and Soderstrom, '15). If for any reason the oxygen supply should fail, the spirometer continues to fall until contact is made with mercury in a second cup, when a bell rings warning the observers that oxygen is needed.

Before it enters the wet-test meter, the oxygen is saturated with water vapor by bubbling through water. As it leaves the meter it is dried by passage through a sulphuric acid tower, and is admitted into the system on the dry side of the Benedict train.

The water circulating system. Distilled water circulates in a closed system constructed wherever possible of block tin. A centrifugal pump forces it up to a tank holding about 27 liters, from which an overflow pipe maintains a constant level and pressure head, which amounts to about 6 feet (2 M.) and suffices to drive up to 2 liters of water per minute through the calorimeter. As it is forced up to the tank, any desired fraction of the water may be diverted through a block tin coil immersed in a bath of ice and water, so that by adjustment of valves the temperature of the water reaching the tank may be regulated to constancy within ½°C. From the tank the water flows through a tin spiral immersed in a stirred 'reheater' bath, which has heating lamps controlled by a sensitive toluene-mercury temperature regulator. The temperature of the water entering the chamber can thus be maintained constant at a desired level, usually between 16 and 17°C., with great accuracy. The constancy of this initial temperature is essential to the accurate measurement of the heat exchange.

The water enters the chamber at the center of the closed glass end, and flows in a Pyrex glass tube parallel to the axis along one side of the chamber. Similar tubes run parallel to this along the top and along the opposite side of the cylindrical chamber. A series of forty Pyrex glass tubes of ⅛ inch (3 mm.) internal diameter arch across (fig. 4) to connect the three larger tubes like the ribs of a boat. The water which enters the system at the back of the chamber is led back from the front end of the tube lying along the opposite side of the chamber, so that the path through each connecting arch offers equal resistance and there is even distribution of flow. An outlet at the highest point, which is at the open end of the chamber, allows trapped bubbles of air to be released. The total calculated absorbing area is about 10 sq.meters. The grid is painted with lampblack to increase its absorbing power.[2]

The water on leaving the calorimeter flows through an automatic measuring device of special design into the lower tank from which it started. None of the common types of liquid flow meters in commercial use can be relied upon to measure accurately rates of flow as small as 1 or 2 liters per minute, especially at so low a head of pressure. Types of meter which weigh the water have been described, a very ingenius one being described as used in the original Atwater-Rosa-Benedict calorimeter (Atwater and Benedict, '05). The meter used here has fewer moving parts and does not depend upon weighing, but upon volume and so avoids errors due to friction. While of comparatively simplicity, it has proved accurate and dependable as well as completely automatic in operation; for this reason details of its design are here given.[3] The meter is shown in figure 5. The two cylindrical cans of about 4 liters' capacity are mounted vertically side by side. At the bottom of each is an outlet controlled by a conical rubber valve, normally held close by gravity. One or other of these valves is opened by rocking of the horizontal arm beneath about a pivot at its center. This arm carries rigidly also a vertical piece which serves to swing the pipe carrying the entering water from one can to the other. In the diagram the water is shown filling the left-hand can (observe meniscus in glass gauge), until finally a hollow cylindrical metal float rises to close a pair of light contacts above it. Current from a dry cell will then actuate a relay (not shown), which throws current supplied by the 110-volt mains, from the solenoid on

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[2] The entire grid was made and installed as one piece by William Pfeiffer, 67 Walzford Rd., Rochester, New York.

[3] Much of the design and its successful construction are due to Mr. L. Hofschneider, technician of this laboratory.

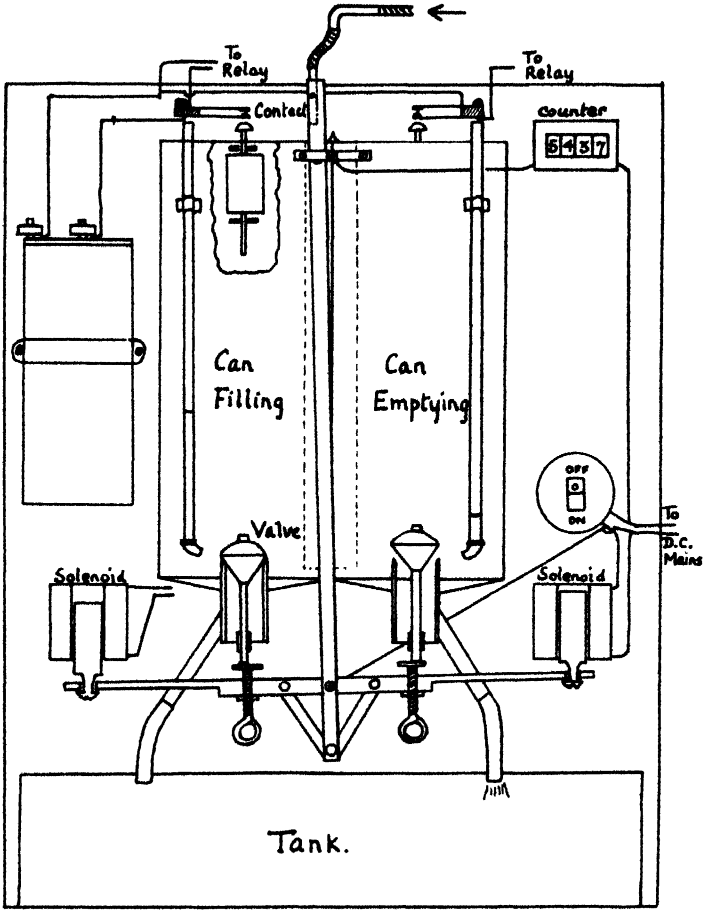

Fig. 5 Recording water meter.

the right-hand side of the rocker arm to that on the left. The rocker swings, lifting the left-hand valve and discharging that can, and swinging the waterpipe to the empty can, the valve which is now closed. As the vertical arm swings, a contact mounted on it causes a 'telephone' counter to record the number of times the cans have emptied. The relay mentioned has an armature pivoted to lie between two electromagnets, one of which is energized by the closing of the set of contacts above each float. Gravity holds the armature in position against either one or the other electromagnet, in which position an arm attached to the armature carries the 110-volt current through one of a pair of mercury cups to the respective solenoid, until a momentary impulse in the other electromagnet pulls it over to lie against that magnet while the can is filling. Thus, although the closing of the contacts by the floats is momentary only, there is always a positive force supplied by one of the solenoids to hold the mechanism in position. The quantity of water delivered by each can, adjusted by the position of the contacts above the floats to be accurately equal, is found to be constant to within 5 cc. (in a total for each can of 4 liters). For the calculation of the heat, the weight of water flowing is required rather than the volume, but since the temperature of the flowing water does not vary by more than a degree centigrade, the density varies so little that it may be assumed constant.

The bed. The bed is a result of trial and error to obtain the greatest comfort in the limited space. It moves upon casters that roll in brass channelling inserted into wooden rails resting in the chamber. The framework of the bed is of oak, the bed itself being made from a canvas hammock. A wide strip of elastic cloth under the back of the subject is found to give additional comfort. When the chamber is open, the bed may be drawn almost completely out on extension rails carried on a trestle which can be placed temporarily in position. The position of the subject in the chamber is seen in figure 4. There is room for him to turn on his side and to make the small movements necessary to his comfort. Free circulation of air all around the subject is provided.

Communication. Communication with the subject is maintained by an electrical signaling system. The cord and key that is held in the hand of the subject are seen descending from the roof of the chamber in figure 4. A similar key outside the box operates a buzzer mounted beneath the track. A simple code of signals suffices for the experimental routine.

THE MEASUREMENT OF TEMPERATURES

The differential water themometers. At the point of entrance and of exit of the water in the chamber, electrical resistance thermometers are immersed in the stream. These are wound of nickel wire to a resistance of 100 ohms. They are enclosed in brass tubing, some 8 inches of which lie axially in the center of the glass tubing carrying the water, and are connected to form a differential thermometer, in a Wheatstone network, an arrangement too well known to need description here. The current includes a zero adjustment by which the bridge may be balanced accurately when the thermometers are at the same temperature, and a sensitivity adjustment for regulating the readings to different ranges of temperature. Standardization is made by adjustment of the current supplied to the bridge to a standard value shown by a milliameter. The difference of temperature between inflowing and outflowing water is then directly recorded on a Cambridge and Paul thread recording galvanometer, the full scale of which represents 1°C.[4] Calibration has been made by immersion of the thermometers in stirred water baths thermostated in the usual way.

A rectal thermometer of the usual electrical resistance type is also connected to the same recorder so that its resistance is recorded on the same chart on alternate minutes with the water difference. For later work, however, this thermometer has not been of sufficient sensitivity, and another, to be described with those used to measure surface temperatures, has been constructed.

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[4] The thermometers, bridge and recorder were supplied especially for the purpose by the Cambridge Instrument Company, of Ossining New York.

The differential air thermometers. The thermometers used to measure the temperature of incoming and outgoing air were originally of a type of construction similar to those used for the water temperatures. It was found, however, that there was a tendency to take up the temperatures of the heavy metal head of the thermometer protruding from the calorimeter box into the room, rather than the temperature of the flowing air. Thermometers of less thermal capacity and thermally insulated from the room were therefore made by winding the resistance coils, covered only by a coating of shellac, on the end of fiber rods pushed axially down the air-pipes at the entrance and exit. The difference of temperature is recorded every minute on a second Cambridge and Paul recording galvanometer. Standardization is here made by the use of a test deflection produced when, for the two resistance thermometers, two fixed resistances differing by a known amount are substituted. A switch is included also by which the resistance of one of the thermometers, the 'exit' thermometer, is compared with a fixed resistance so that the absolute value of the temperature is recorded instead of the temperature difference. This is used, during the taking of 'residuals' at the conclusion of each period, to find the temperature of the chamber air.

The gradient and wall thermometers. In figure 6 the heat insulation is removed from one section of the glass wall of the chamber to show the resistance thermometer that is wound spirally on the outside of the cylindrical wall to give automatically the average temperature over its surface. Nickel wire of no. 40 gauge is attached by sewing to the long strip of cloth shown, there being about 170 feet of wire in all and a total resistance of over 1000 ohms. The insulation consists of a blanket 1½ inches (3.75 cm.) thick, of 'dry zero,' a kapok-like material that has very constant insulating properties. Outside the blanket is a second resistance thermometer in a strip of cloth (shown to the left) spirally covering the cylindrical area. The difference of temperature between these two thermometers gives the thermal gradient to which the escape of heat through the insulation is proportional. The second recording galvanometer is double and records this gradient every 2 minutes. Alternate minutes an automatic switching device driven by the same clockwork, compares the inner, wall thermometer with a fixed resistance coil, to record the wall temperature. The recording galvanometers are seen on the shelf in figure 7 with the bridges and batteries below.

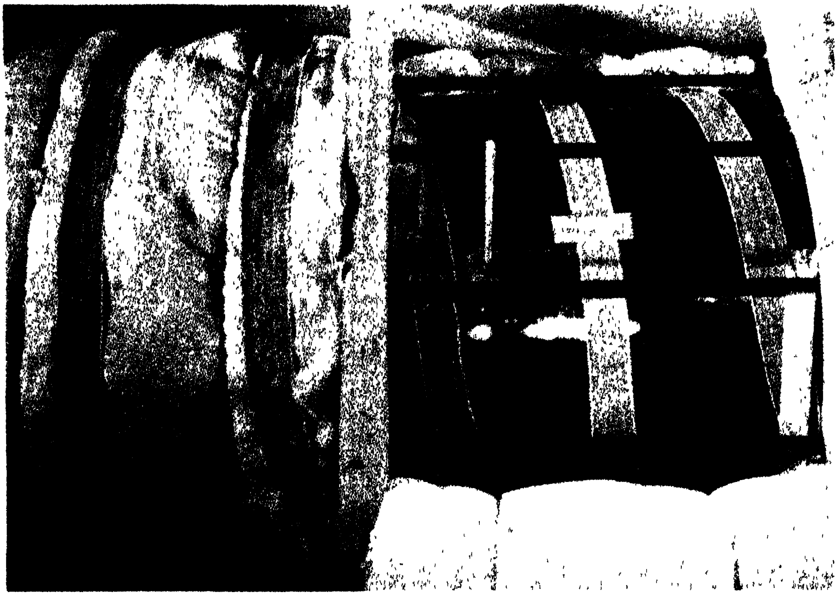

Fig. 6 Insulation of chamber and gradient thermometers.

Rectal and surface thermometers. In order that the calculation of heat stored or lost by the body of the subject may be made as accurately as possible, the average temperatures over areas of the body surfaces have been measured as well as the rectal temperature in all of the later experiments. The technic is that already described by Burton ('34), in which resistance thermometers are made by sewing nickel wire into areas of light gauze cloth which are held close to the skin. One of these thermometers is worn over the surface of the trunk, one over the lower leg and one over the forearm of the subject, the average temperature for other parts of the surface being estimated by interpolation from these. Clothing may be worn over the gauze thermometers, so that conditions are as normal as possible. The rectal thermometer is wound of the same no. 40 gauge nickel wire on the end of a hard rubber rod of ¼ inch (0.62 cm.) diameter. A spherical swelling 2 inches (5 cm.) from the end of the rod serves to keep the thermometer comfortably in position. The coil and rod are dipped and baked with three coatings of transparent bakelite, which is found to be the most satisfactory of any coatings tried. Leads from the thermometers leave the chamber by a

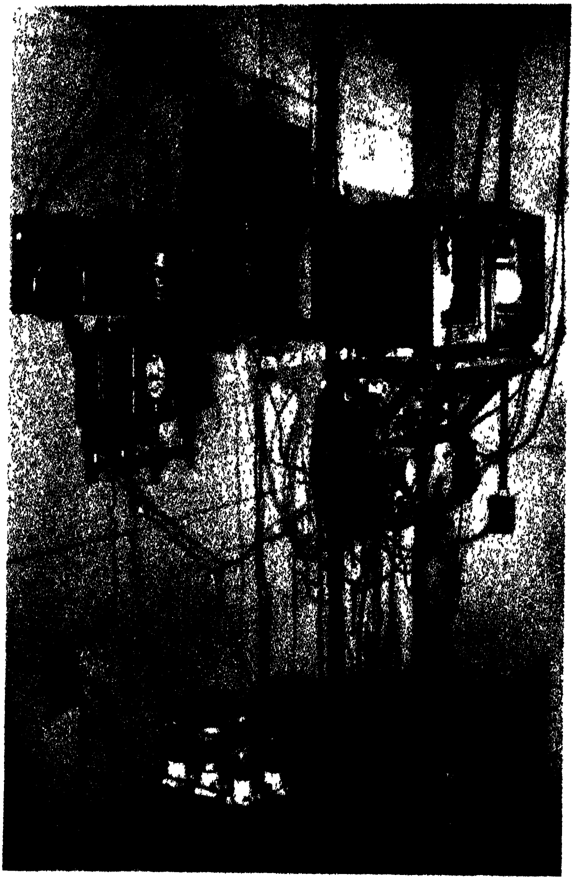

Fig. 7 Electrical recording instruments.

hole in the upper surface to be connected to the multiple bridge shown diagrammatically in figure 8. A switch arm 'S' swings over a series of mercury cups 'C,' by which in turn each of the thermometers is brought into the Wheatstone network with its appropriate balancing resistance. In the

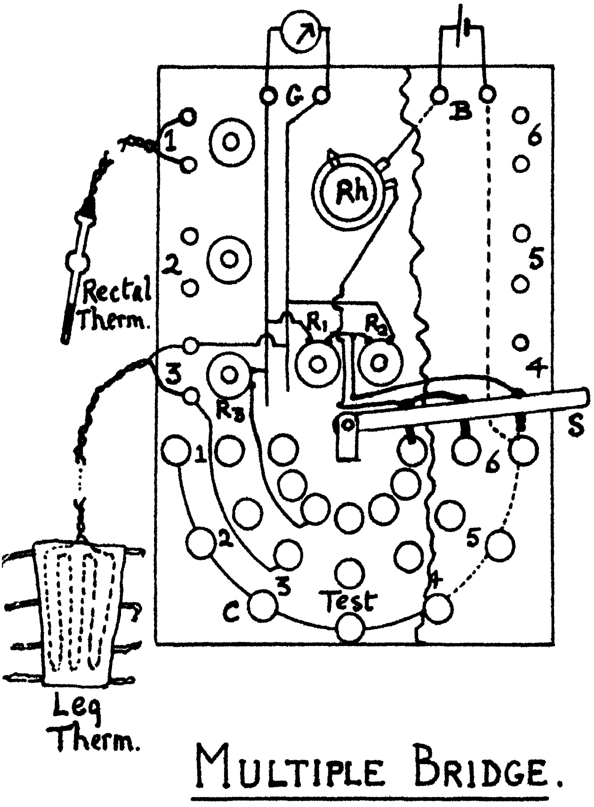

Fig. 8 Multiple bridge for body temperature measurements.

diagram, half of the bridge cover is shown cut away to reveal the essential connections to one of the thermometers, the leg thermometer, only. R_3 is the fixed coil balancing this thermometer; R_1 and R_2 are the 'ratio coils' of the bridge. The deflection of the galvanometer gives directly the temperature of the thermometer on reference to a calibration chart made when the thermometers are immersed in a metal container in stirred constant temperature baths. In the central position the switch connects, instead of a thermometer and its balancing resistance, two fixed resistances, to give a 'test deflection.' Adjustment of this to a standard value by the rheostat Rh ensures accuracy of the readings. Current from a 1½ volt dry cell is sufficient to give a sensitivity of 3 cm. on the scale to a degree centigrade, which is ample for physiological experiments. The temperatures of all the four thermometers can be read in the space of less than a minute. In routine calorimeter experiments they are recorded every 5 minutes, though for the calculation of the direct heat the temperature at the beginning and end of a period are the only ones needed.

Electrical heat checks. The calculation of the total heat received by the apparatus requires a knowledge of the number of Calories per hour escaping through the insulation for each value of the gradient across it, and also of the number of Calories that are absorbed for each degree rise of the temperature of the wall—the hydrothermal equivalent of the apparatus. Both of these are found by a series of experiments in which energy of known amount is supplied in the chamber by an 'electrical man.' This is a wooden framework on which resistance wire is strung and which is covered with asbestos cloth to give an area and shape similar to that of a man. The energy supplied to this is read from a kilowatt-hour meter. (See in fig. 7 on the wall in the foreground.) The rotating disc in the meter carries a light silvered coverslip which, once per revolution, reflects the light of a lamp mounted above to a photronic cell, which through a Weston signal relay system, actuates a counter. Multiplication of the number of revolutions this counter records in a period by a factor gives the number of Calories supplied to the chamber. The meter has been calibrated against standard voltmeters and ammeters through the courtesy of the General Railway Signal Company of Rochester.

In the check experiments, the rate of water flow and of energy supply is adjusted until thermal equilibrium is reached, so that in an hour period there is no recorded change of temperature of the wall, and thus no item of heat to be reckoned as entering or leaving the apparatus itself on that score. The heat entering the water, the air, and that lost by evaporation are calculated. The discrepancy between the total and the heat electrically supplied gives the heat that has escaped through the insulation of the calorimeter. The rate of the heat loss, or gain, is then plotted against the average gradient of the period, to give the curve used in future experiments to calculate the heat loss from the average gradient. Since, in the determinations of this curve, all the sources of heat measurement are present that occur when human calorimetery is studied, the deviation of the points from a smooth curve is a measure of the errors in the measurement of heat by the apparatus. The errors are found to be not greater than 1 or 2 Calories per hour. In practice, the temperature of the calorimeter room is set to such a value that the gradient of temperature across the insulation is small, and the item of heat lost or gained by this route is a small one.

For the determination of the hydrothermal equivalent, the experiment is continued after the electrical energy has been shut off. The records of the temperatures are then as in figure 9 (where the three charts are combined and scales for wall temperature, gradient and cooling of air are on the left-hand side, that of the water difference on the right). The rise of temperature of the flowing water does not fall immediately to zero, since the water continues to receive heat from the cooling chamber. A tangent drawn to the curve of falling wall temperature at a point such as that of the dotted arrows gives the rate of fall of temperature in degrees per minute. The values of the temperatures on the other curves allow the number of Calories per minute given off to be calculated. Division gives the hydrothermal equivalent. An example is given in table 1. The constancy of the results of the thirteen determinations given at the foot of the table is an indication of the accuracy of the heat measurement. Since in an ordinary experiment the change of wall temperature is rarely more than 0.1°C., the item of the heat gain or loss of the glass chamber itself is known with considerable accuracy. The above method is considered much better than estimation of the hydrothermal equivalent from weight and specific heat of the chamber.

Residual analysis. It is necessary to know what change has occurred during a period in the carbon dioxide, oxygen and water vapor contents of the chamber. To this end, at the beginning and end of each period, a sample of the circulating air is drawn off, passed through weighed absorbing U-tubes, and through a gas meter until 6 liters has passed, then back into the system. The temperature and pressure of the air in the chamber must also be known with accuracy, since a change of temperature of about 0.75°C. or a pressure of about 2.0 mm. produces a change of 1 liter in volume (total volume is about 560 liters). The pressure is, of course, read from a barometer, the temperature is taken as the average of three temperatures,[5] that of the ingoing, of the outgoing air, and of

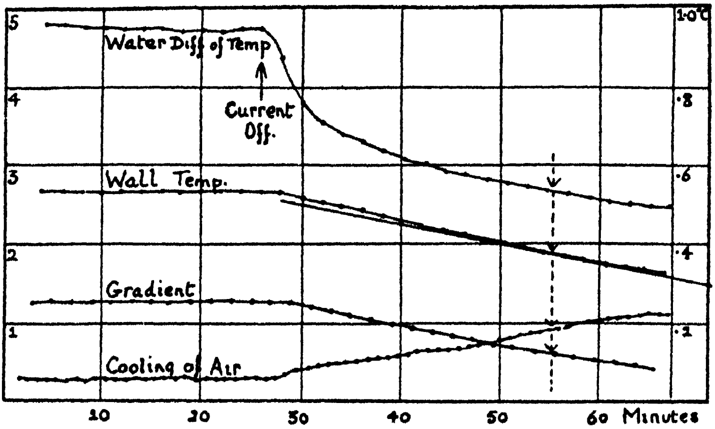

Fig. 9 Determination of hydrothermal equivalent.

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[5] As this paper goes to press an improvement in recording the fluctuations of temperature of the chamber air has been made by employing several electrical resistance air thermometers in series.

a thermometer (seen in fig. 6), entering the top of the chamber above the subject.

Partition of the heat measured. Table 2 shows the relative magnitude of the various items that normally occur in the

TABLE 1

Calculation of hydrothermal equivalent

Rate of fall of wall temperature	0.0385°C./min.
Temperature difference of water	0.530°C.
Rate of flow of water	1.478 kilos./min.
Heat entering water	0.7848 Cals./min.
Cooling of air	2.11°C.
Rate of flow of air	34 liters/min.
Heat from air	0.0213 Cals./min.
Gradient across insulation	1.62 divs.
Heat into room	0.0133 Cals./min.
Total heat from wall	$= 0.7848 + 0.0133 - 0.0213$
	$= 0.7768$ Cals./min.
Hydrothermal equivalent	$= \frac{0.7768 \text{ Cals./min.}}{0.0385°\text{C/.min.}} = 20.2 \text{ Cals./°C.}$

Results 22.1, 21.8, 22.3, 19.5, 23.0, 22.5, 22.1, 23.1, 21.9, 20.1, 23.5, 23.9, 20.2. Average H.T.E. 22.1 ± 1.5 Cals./°C.

TABLE 2

Partition of heat measurement. Experiment July 19, 33

	PERIOD I	PERIOD II	UNIT
Weight of water flowing	90.45	92.20	Kilos.
Average rise of temperature	0.712	0.707	°C.
Heat into water	64.4	65.2	Cals.
Average gradient	2.11	2.13	Divs.
Heat into room	2.95	3.2	Cals.
Rise of temperature of wall	0.162	0.015	°C.
Heat into wall	3.8	0.35	Cals.
Average rise of temperature of air	— 1.97	— 1.97	°C.
Heat into air	— 1.55	— 1.55	Cals.
Total heat by radiation and convection	69.6	67.2	Cals.
Water evaporated	21.8	24.0	Gms.
Heat by evaporation	12.55	13.8	Cals.
Rise of rectal temperature	— 0.16	— 0.05	°C.
Heat stored by body	— 9.0	— 2.8	Cals.
Total direct heat	*73.15*	*78.2*	Cals.
Indirect heat	*74.9*	*78.7*	Cals.

total direct heat, of which some 80 per cent is usually in the flowing water. For the heat lost by evaporation, the figure 0.580 Calories per gram of water evaporated is used. The latent heat of vaporization of water at 20°C. is 585.84 Calories; at 30°C. 580.04 Calories. The value of 586 Calories, correct for 20°C., has been used by some workers. The evaporation of water from the human skin takes place at a temperature certainly above 30°C., the average skin temperature being about 33°C. The value 0.580 kilocalorie per gram of water evaporated is therefore used. The water collected in the acid bottles of the train may not truly represent the amount evaporated by the subject, since water may accumulate or be lost from the calorimeter walls or from the bed. It does accurately give, however, the heat removed from the calorimeter by evaporation for the calculation of the total heat received by the apparatus. There is, of course, a small correction due to cooling of the water vapor from the temperature of the skin to the temperature and relative humidity of the chamber.

The question of which figure should be used in the calculation, one of academic rather than practical interest, since the final differences are so small, is a complex one upon which we have encountered considerable difference of opinion. Without doubt, if we confine ourselves to consideration of the heat lost from the body surface by evaporation, the figure for the latent heat appropriate to the average skin temperature should be used. The balance of the heat lost by the body is through radiation, convection and conduction, but the amount of this portion of the heat loss is not accurately equal to the heat received and recorded by the absorbing system of the calorimeter, since the latter includes a small item of heat given up by the water vapor cooling to the final state of temperature and humidity in which it leaves the chamber. Thus in our partition of heat losses in table 2, the loss by evaporation is correct, but the loss by radiation and convection is slightly in error. (A calculation shows that the error in the case cited does not exceed 0.1 Calorie). Our total heat loss is also in error by the same amount, being the sum of an accurate partition to evaporation, and an inaccurate partition to radiation and convection.

Other workers, who are perhaps more interested in the accuracy of the total than in its component parts, retain the inaccurate partition to the radiation and convection and bring the total to supposed accuracy by altering the evaporation component, using the latent heat appropriate to chamber temperature instead of skin temperature and a correction for the cooling of the water lost by the body from body temperature to chamber temperature. Their total heat loss is then (supposedly) correct, but is composed of two components, neither of which can be accurately partitioned to evaporation or to radiation and convection. The total is not really accurate, since no account has been taken of the heat exchange in the expansion of the saturated vapor at chamber temperature to the relative humidity of the chamber—a not inconsiderable correction. Of the three quantities, the total heat loss and its two components, none can be calculated accurately without consideration of the relative humidity of the chamber. Our method has at least the virtue of giving one of the components, the loss of heat by evaporation from the skin, in an unequivocable way.

The heat stored or lost by the body of the subject in changing its average temperature may amount to a very large item, the uncertainty of our knowledge of which is discussed in the following paper.

Alcohol checks. Proof of the absence of gas leaks and of the accuracy of the calorimeter is sought by the normal method of burning 94 per cent ethyl alcohol in the chamber at frequent intervals between human experiments. Two small flames fed by asbestos wicks in a glass Y-piece, were found to be much steadier than a single flame of the same capacity. The burner is connected by rubber tubing to a regulating apparatus fixed to the outside of the calorimeter box. By the opening or closing of two stopcocks the alcohol is siphoned alternately from one or the other of two bottles supported on blocks of wood. At the end of an hour's period, by turning the taps the supply is shifted to the other bottle, while the blocks beneath the first are removed so that it may be lowered, removed, weighed and replaced ready for a further period. In this way the weight of alcohol burned in the actual period of experiment may be directly found by weighing for a number of successive periods. The steadiness of the flames is ensured by keeping the level of alcohol constant by the insertion of thin cards under the bottles when needed.

In table 3 the results of all alcohol check periods made during a space of more than a year are given. Those periods in which there was an obvious leak, shown by the very poor value of the R.Q., are the only ones omitted. The close agreement between the averages of the direct heat from the calorimetry and the indirect heat from the amount of oxygen consumed, amounting to 0.17 per cent, shows that the systematic error in the heat measurement is very small.[6] The average of the differences between direct and indirect heats in individual hour periods is 2.35 Calories, or 2.9 per cent, and, as the equality of the totals shows, is as likely to be positive as negative. This 2.9 per cent and not the 0.17 per cent, is the correct average instrumental error of the apparatus in comparing direct and indirect heat. The average error in measurement of either direct or indirect heat alone must be less than this.

It is interesting to find that the systematic error' (percentage difference between totals) is exactly the same in this series as was given by Gephart and DuBois ('15) for the first series on human subjects run with the Sage calorimeter. Coincidently also the first report of alcohol checks with the Sage calorimeter included nineteen periods (Riche and Soderstrom, '15) as does the present report. The percentage errors found have not been calculated on quite the same basis in this report as in that of Riche and Soderstrom, but they are obviously very similar. For example, in the Sage calorimeter the average total error for oxygen in the alcohol checks was —1.69 per cent; in the present series it is —1.4 per cent. The average individual error for the difference between theoretical

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[6] I.e., there is no tendency to give regularly either too low or too high a value for either measurement. The mode of the difference lies at + 0.142 Calorie on the side of the direct heat.

TABLE 3

Alcohol heat checks

DATE	ALCOHOLISM CONSUMPTION IN GRAMS	L.O.$_2$ THEORY	FOUND L.O.$_2$	HEAT THEORY	HEAT FOUND Direct	HEAT FOUND Indirect	R.Q.
1933							
May 4					82.4	81.4	0.672
May 9	12.75	18.12	18.47	88.7	87.8	89.6	0.628
	10.88	15.40	15.55	75.8	73.0	76.0	0.722
May 15	8.47	11.50	10.93	56.1	56.8	53.1	0.687
May 19	11.48	16.37	15.67	79.5	80.0	76.1	0.674
	10.76	15.40	15.09	76.2	72.0	73.3	0.671
	10.30	14.74	14.73	71.4	71.6	71.6	0.653
June 27	15.17	21.71	20.80	105.1	101.8	109.1	0.677
	13.21	18.90	18.64	91.6	86.5	90.4	0.650
July 14	13.31	19.02	18.44	92.3	88.2	89.6	0.662
	14.90	21.34	21.53	103.2	104.2	104.2	0.658
1934							
January 5	12.10	17.30	17.51	83.9	87.1	84.9	0.665
	13.06	18.70	18.70	90.4	89.9	91.5	0.654
January 15	11.32	16.20	15.48	78.5	76.4	75.1	0.659
	11.31	16.19	14.96	78.6	77.1	72.5	0.670
February 2	10.29	14.55	14.52	71.4	73.0	70.4	0.659
	11.23	15.88	16.24	77.9	77.2	78.8	0.656
May 25	11.98	16.23	15.92	78.6	78.9	77.2	0.670
	12.10	16.45	16.65	79.6	79.0	80.8	0.665
Averages	11.92	16.89	16.66	82.2	81.205	81.34	0.666
Totals		304.00	299.87	1478.8	1542.9	1545.6	
Difference		—1.4%			—1.3%	+ 0.17%	
Average of individual differences					± 2.25%	± 2.9%	± 1.95%
Coefficient of variation		$\frac{\sigma \times 100}{\text{mean}}$			2.8%	3.6%	2.5%

heat and 'heat found' (presumably direct heat is meant) in the alcohol checks for the Sage calorimeter was 2.51 per cent without regard to sign; in the present series the same error is 2.25 per cent. The Sage workers do not give the average individual difference between direct and indirect heat, with which the error of 2.9 per cent given above could be compared. The average respiratory quotient is exactly theoretical, but the average difference or scatter between the individual measurement and theory is ± 1.95 per cent.

Many investigators now prefer to see variations expressed as standard deviations from the mean and the coefficient of variation as a percentage which the standard deviation is of the mean. The latter are given also in table 3. These surely must be regarded as highly satisfactory.

Accuracy in human calorimetry. With human subjects two additional sources of error arise. The first, which has been mentioned, is that the indirect heat can be calculated from the oxygen consumption only by assuming values for the Calories per liter of oxygen that are accurate for a limited group of reactions taking place in the body, namely, the oxidations of fat, protein and carbohydrate. The indirect heat may then be in error by an unknown amount, which might conceivably be considerable in short periods.

It is still true that the calorimeter measures heat with an average error of less than 2.9 per cent, but what is measured is the heat eliminated from the subject. This is not, as it is with an alcohol flame, equal necessarily to the heat produced in the same period, as considerable amounts of heat may be stored or lost by rise or fall of body temperature. The body temperature can only be measured at a limited number of points (in the past one only, the rectal temperature has been used) and thus this item of the heat exchange has a large possible error. The result is that the average difference between direct and indirect heat in hourly periods is, with human subjects, of the order of 7 or 8 per cent when the rectal temperature alone is used. By using the surface temperatures also, in the manner explained in the following paper, the average error has been reduced to 5½ per cent.

The increased error cannot, however, fairly be considered as characteristic of the respiration calorimeter, the function of which is to measure the respiratory exchange and the heat given off by a subject. Each of these types of measurement it makes with an average error not greater than 2.9 per cent.

SUMMARY

A respiration calorimeter is described in which the measurement of direct heat has many automatic features. The respiration chamber is a cylinder of Pyrex glass through which air circulates in a closed circuit including two interchangeable Benedict trains of absorbers. Oxygen is admitted automatically as it is consumed and is measured on a wet test meter. Cold water, brought initially to a constant temperature, passes through a grid of pyrex tubes to carry away most of the heat. The temperatures of inflowing and outflowing water and air, and the temperature of the glass wall are electrically measured and automatically recorded every minute. Water flow and air flow are recorded by meters of special design.

The calorimeter is not adiabatic, the escape of heat through the insulation around the walls being measured by the thermal gradient set up across it, which is electrically recorded by resistance thermometers. Calibration with electrical input of heat gives the heat flow for a given gradient and also the hydrothermal equivalent (22.1 Calories per degree).

In a series of alcohol checks, the totals of direct and indirect heats differ by 0.17 per cent, while the average difference in individual hour periods is 2.9 per cent. In human calorimetry, the average difference is more, but by the use of rectal and surface temperatures measured by resistance thermometers, it has been brought to 5.5 per cent.

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HUMAN CALORIMETRY

II. THE AVERAGE TEMPERATURE OF THE TISSUES OF THE BODY [1]

ALAN C. BURTON
Department of Vital Economics, University of Rochester, Rochester, N. Y.

THREE FIGURES

(Received for publication August 2, 1934)

It has been generally tacitly assumed that the average temperature of all the tissues of the human body is very close to the 37°C. or thereabouts, registered by a rectal or an oral thermometer. It is known, however, that the surface of the body is at a temperature considerably lower than this. In normal surroundings, say at 25°C., the average temperature for the whole skin surface of a lightly clothed man will be in the neighborhood of 33°C., the average being a mean of widely different temperatures at different parts of the body surface. At the feet the temperature may be as low as 27°C., while on the abdomen it may approach 35 or 36°C. The temperature, of course, rises as we advance from the skin to the interior. The experiments of Bazett and McGlone ('27) showed that in the forearm and thigh, the constant internal temperature was not reached until a depth of about 2.5 cm. was reached. Those of Benedict and Slack ('10), on the temperatures at different depths in the rectum and vagina, showed that constancy was not reached until a depth of 5 to 7 cm. In the view of these facts, the assumption that the average temperature is close to 37°C. cannot be made without investigation.

In direct calorimetry the knowledge of the true average temperature of the body is of great importance. A calorimeter

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[1] This work has been aided by a grant from the Rockefeller Foundation.

measures the heat eliminated from the body, and if from this the heat production in the same period is to be inferred, the heat stored or lost by the body in changing its average temperature must be known. How large this item in the heat balance may be, and how great is the uncertainty in its calculation has been fully recognized by workers in calorimetry. It is discussed by Benedict and Carpenter ('10). It may be pointed out that in the case of a 70 kg., man, a rise of 0.2°C. in body temperature, not uncommonly occurring in the space of an hour, corresponds to a storage of nearly 12 kilocalories of heat in the body. This would amount to about 15 per cent of the normal basal heat production of the man in that period, and there might be a 15 per cent difference between direct and indirect heats. It was recognized by these authors that a research was very necessary on the topographical distribution of temperature in the body and its fluctuations, and that until it was made, it had to be assumed that fluctuations in body temperature as measured by a rectal thermometer are a true measure of those of the true average temperature of the whole body.

Benedict and Slack ('11) made simultaneous measurements of the temperatures in the rectum, the vagina, and in natural and artificial cavities, such as the axilla, the groin, and between the closed hands. They found a fairly close parallelism between the fluctuations of the temperatures of these points and concluded that "aside from the skin temperature, a rise or fall in rectal temperature is accompanied by a corresponding rise or fall in temperature of all other parts of the body." The use of the rectal temperature alone therefore appeared justified.

There is strong evidence, however, that grave errors exist in making the calculation of heat storage or loss from the rectal temperature alone. Thus it is claimed that the respiration calorimeter at the Russell Sage Institute of Pathology measures oxygen consumption and heat with an average error of 1.6 per cent and 0.9 per cent, respectively (Riche and Soderstrom, '15). Yet with normal subjects, all that could be said was that "of a total of 28 periods in 17 the difference between direct and indirect heats was less than 5 per cent and only 6 showed more than 10 per cent difference." In the case of our own calorimeter in Rochester, the difference between direct and indirect heats in alcohol checks is on the average 2.9 per cent, while with human subjects, using rectal temperature alone for calculation, the average discrepancy is about 8 per cent, and cases occur where it is as much as 20 per cent. There is also the fact that changes in rectal temperature occur often which are so rapid that it is not conceivable that corresponding changes could have occurred in the whole body, the extra heat generation necessary for such a total body temperature rise being too great. Also it is well known that in typhoid and malarial fevers the rectal temperature may fall, while the surface temperature rises and vice versa. In the same series of papers, Gephart and DuBois ('15) made an attempt to use the surface temperatures measured at three or four points on the surface to improve the agreement, but with little success.

With the respiration calorimeter now in operation at Rochester, New York (Murlin and Burton, '35), data on a series of 1 hour calorimetry periods are available, in which in addition to the rectal temperature average temperatures of large areas of the surface of the body have been recorded by the method of resistance thermometry described by Burton ('34 a). These make possible a statistical examination of the possibility of using the surface temperatures as well as rectal temperature in calculating the average temperature of the body.

Theoretical considerations. The temperatures that Benedict and Slack excluded from their generalization, namely, the skin temperature, are in all probability of much greater significance than those they selected in estimating the average temperature of the body. The location of their thermometers was such that, naturally or artifically, the outflow of heat from these points was greatly reduced. In these circumstances, the temperature must approach that of the interior of the body, as shown by the fact that all of their recorded temperatures lay above 36°C. What their results demonstrate is that the rectal temperature gives a reliable estimate of the temperature of the deep tissues of the body.

The magnitude of that portion of the body that lies within 2.5 cm. of the surface, the distance to which the gradient certainly extends, is surprisingly high, as a simple geometrical calculation shows. Suppose that the limbs, as the lower legs, are treated as truncated cones of mean diameter D cm. and of uniform density. The percentage of the volume that lies within 2.5 cm. of the surface is then given by the formula—

$$100\left(1-\frac{(D-5)^2}{D^2}\right)$$

In table 1 the calculation has been made from measurements on the author. The second column gives the mean diameter, the third column the percentage of the part of the body that is within 2.5 cm. of the surface. The head is treated as a sphere, for which the formula is—

TABLE 1

Calculation of proportion of body affected by surface temperature

PART	MEAN DIAMETRE, CM.	PER CENT PART NEAR SURFACE	PART AS PER CENT OF WHOLE	PER CENT OF WHOLE NEAR SURFACE
Feet	....	80.0	3.6	2.9
Lower legs	9.3	78.5	10.5	8.3
Upper legs	14.4	57.6	23.2	13.3
Trunk	26.3	34.5	42.7	14.7
Upper arms	8.9	81.0	6.7	5.4
Lower arms	7.3	90.0	4.6	4.1
Hands	....	80.0	1.7	1.4
Head	18.0	62.0	7.0	4.3
	Total percentage of body near surface			54.4

$$100\left(1-\frac{(D-5)^3}{D^3}\right)$$

The next column gives, from the figures of Braune and Fischer (1889), the weight, or volume, of the limb as a percentage of the whole body. The last column is the result of multiplying together the third and fourth columns and gives the percentage which that peripheral tissue forms of the whole body. The total of the last column states the surprising fact that 54.4 per cent of the body of the author is within 2.5 cm. of the surface. The calculation has little significance except to emphasize the fact that a very large proportion of the whole body is at a temperature considerably lower than the internal temperature. For a small subject a similar calculation gave 58 per cent, for obese subjects it might be less than 50 per cent. Obviously, the temperature of the surface must be given considerable weight in calculating the average temperature of the whole body.

The manner in which the internal and the surface temperatures are to be weighted in finding the average temperature of these peripheral tissues depends upon the nature of the thermal gradients existing in them. The work of both Bazett and McGlone ('27) and of Benedict and Slack ('11) shows that the distribution of temperature is not linear, but in general parabolic, the gradient being steeper near the surface, a form of distribution that agrees with theoretical predictions for a cylinder in which heat is being uniformly generated (Burton, '34 b). Simple cases may be taken assuming different temperature distributions, to illustrate how the average temperature T_A depends on the internal temperature T_i and surface temperature T_s. a) For a plane slab with linear distribution of temperature, of course—

$$T_A = .5\,T_i + .5\,T_s$$

b) For a cylinder with linear distribution of temperature (a case not physically possible)—

$$T_A = .33\,T_i + .67\,T_s$$

c) For a cylinder in which the distribution of temperature is parabolic throughout—

$$T_A = .5\,T_i + .5\,T_s$$

Once more the average is the arithmetic mean of the internal and external temperature. This is evidently the best way to calculate the average temperature of the limbs, being theoretically sound as well as the simplest possible. For the trunk, however, this method of averaging might be thought to give undue weight to the surface temperature, since here there is a large proportion (65 per cent or so) that lies more than an inch from the surface. We might assume that the gradient extends to a depth of 1 inch and that inside there is uniform temperature. This distribution could arise only if in the inner region there were very effective transfer of heat, or if within it there was a relatively small amount of heat generated. The first of these conditions is probably true, the second is hardly correct, since the visceral organs are large generators of heat. It is more likely that a parabolic distribution holds to greater depths than 1 inch. The temperature of the stomach is known to be slightly higher (0.1°C.) than that of the rectum (Rancken and Tigerstedt, '08).

A calculation has been made to find the formula for the average temperature, assuming that the distribution is parabolic up to a depth of 2 inches from the surface, and uniform at the temperature T_i inside. The result is—

$$T_A = .8\,T_i + .2\,T_s;$$

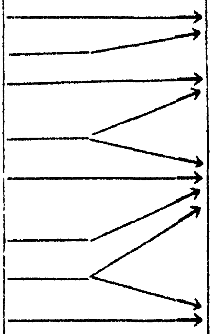
using 1 inch as the critical depth, it would be .89 T_i plus .11 T_s; using 3 inch, .71 T_i plus .29 T_s. The figures .8 T_i plus .2 T_s are used for the trunk in the next calculation, which consists in taking the formula for the average temperature of each part of the body, assuming in each case that the internal temperature is the rectal temperature T_R, and multiplying by the fraction that the part forms of the weight of the whole body to get the contribution of that part to the final formula. Addition of the contributions for all the parts gives a final formula involving all the temperatures, T_1, T_2, T_3 etc., measured, of the form:

$$T_A = A_1\,T_1 + A_2\,T_2 + A_3\,T_3 + \ldots.$$

Table 2 gives, in the second column, the calculated values of the coefficients A_1, A_2, appropriate to the temperatures measured at the various places listed in the first column. If it is not possible or desirable to measure temperatures at all the places mentioned, the formula may be simplified for use with a smaller number of temperature measurements. For instance, the surface temperature of the upper arm may be assumed to be the mean of those of the lower arm and of the trunk. Its coefficient (0.033) is then divided equally between the coefficients for the trunk and for the lower arm, as shown by the arrows in the table. (Mathematically, if, in the formula T_2 is put equal to $\frac{T_1+T_3}{2}$, there results:

$$T_A = (A_1 + \tfrac{1}{2} A_2)\, T_1 + (A_3 + \tfrac{1}{2} A_2)\, T_3 + \ldots .)$$

TABLE 2

POSITION WHERE TEMPERATURE IS MEASURED	COEFFICIENT IN FORMULA	REPARTITION OF COEFFICIENTS	COEFFICIENT IN SIMPLIFIED FORMULA	TEMPERATURES USED IN SIMPLIFIED FORMULA
Rectal temperature	.59		.64	Rectal
Oral temperature	.05			
Surface of lower leg	.07		.13	Surface of lower leg
Surface of upper leg	.12			
Surface of trunk	.09		.18	Surface of trunk
Surface of head	.02			
Surface of upper arm	.03			
Surface of lower arm	.03		.05	Surface of lower arm

The simplified formula in the third column applies when the surface temperatures on the trunk, on the lower leg, and on the lower arm are used. It may be recast into the form—

$$T_A = .64\, T_R + .36\, T_S$$

where T_s is an average surface temperature given by—

$$T_s = .50\, T_1 + .36\, T_2 + .14\, T_3$$

where T_1 equals average temperature on surface of trunk; T_2 equals average temperature on lower leg; T_3 equals average temperature on lower arm. It is seen that the rectal temperature carries 64 per cent of the average.

This calculation may appear to be based upon so many assumptions that it can command little confidence, but it is found that quite considerable changes may be made in those assumptions without changing greatly the resulting formula. It is remarkable that this theoretical formula, which was calculated before any experimental verification had been made, so nearly approximates to the one found purely by experiment. It suggests that the human body may be more a fit subject for mathematical equations than has been realized.

Experimental determination. The material for the experimental determination of the formula for 'mixing' the rectal and surface temperatures was provided by a series of experiments in the respiration calorimeter upon nine subjects, seven of them male, two female, providing the necessary data of forty 1-hour periods. In nineteen of these periods the subjects were in the basal state, the rest of the periods followed 2 and 3 hours after the ingestion of a carbohydrate or a meat meal. The details of the experiments and the bearing of the results on the effect of ingestion of food are discussed in another paper (Burton and Murlin, '35). The data are thus of a character taken under conditions so diverse that the resulting conclusion as to the average temperature of the body has an 'omnibus' value, and is applicable to calorimetry in general. A result of general application was desired rather than one, perhaps of more accuracy, but applying only to a given subject under a given set of conditions.

Given a sufficient number of determinations by the respiration calorimeter of the heat stored or lost by the body in hour periods, and of the changes in rectal temperature and in surface temperature occurring in the same periods, it is obviously possible to determine experimentally the best coefficient for the rectal and surface temperatures. Assume that the formula is of the type—

$$T_A = aT_R + bT_S$$

The consideration that if the rectal temperature and the surface temperature each rise by, say 1°C., the average temperature must also rise by 1°C., leads to the conclusion a plus b equals 1; or b equals (1 — a). The equation then can be written:

$$T_A = aT_R + (1 - a)\ T_S.$$

If in any period the changes in the rectal, surface and average temperatures are—

$$\Delta\ T_R,\ \Delta\ T_S \text{ and } \Delta\ T_A$$

then—

$$\Delta\ T_A = a\ \Delta\ T_R + (1 - a)\ \Delta\ T_S$$

The change in the average temperature, $\Delta\ T_A$, is found by dividing the quantity of heat stored or lost by the body in the period, equal to the difference between indirect and direct heats, by the thermal capacity of the body. The latter is assumed to be the product of the weight and an average specific heat, taken as 0.83 by general custom, i.e.,

$$\Delta\ T_A = \frac{\text{Indirect heat—Direct heat}}{\text{Weight} \times 0.83}$$

The problem is then to find the value of the coefficient 'a' which statistically makes the equation most nearly true for the greatest number of cases. The value $\Delta\ T_S$ is calculated from the three surface temperatures measured, namely, that over the trunk, over the lower leg, and over the forearm, by the formula: T_S equals .50 T_1 plus .36 T_2 plus .14 T_3, which weights the respective temperatures according to the mass of tissue beneath them. (Weighting the temperatures according to area would give relatively more weight to the leg and forearm.)

From the results of any given period, the value of 'a' best fitted to that set of values can be found from the relation—

$$\frac{\Delta\ T_A - \Delta\ T_S}{\Delta\ T_R - \Delta\ T_S}$$

It might be thought that this ratio should be calculated for each period and the resulting values of 'a' averaged. This, however, would give undue weight to those determinations that are least able to give a reliable value of the coefficient. For suppose that in a period, the change in rectal and in surface temperatures, though both considerable, are equal.

Then, no matter how the two are weighted in the average (i.e., no matter what the value of 'a' chosen), the resulting change in average temperature is the same quantity. Such an experiment can give no information as to the value of the 'mixing coefficient' 'a.' Mathematically, the same thing is stated when we observe that if the denominator $(T_R - T_S)$ is a small quantity, very slight changes in its value, perhaps due to the errors of measurement of the temperatures, produce very large differences in the value of 'a.' The theory of least squares indicates that the best value of 'a' is obtained by the formula—

$$a = \frac{\Sigma XY}{\Sigma Y^2}$$

where X equals $(T_A - T_S)$ and Y equals $(T_R - T_S)$. In the sums here, those experiments in which the quantity Y is small are automatically given the least weight.

Since the quantity ΔT_A obtained from the difference between indirect and direct calorimetry, is subject to the errors of the respiration calorimeter, the problem is essentially soluble only statistically. It was therefore imperative, as in all statistical work, that all results obtained should be included unless there were reasons for rejection other than that the values of the coefficient 'a' appeared to diverge from the average. Such valid reasons were considered to be wildly improbable values of the R.Q., indicating that the oxygen consumption might be incorrectly measured, or obviously incorrect temperature measurements which fluctuated abnormally. In the whole series, only one set of three periods on one subject has been rejected for such reasons. In table 3 the results of the forty periods are tabulated. The last three columns record the progress of the sums ΣY^2 and ΣXY and of the statistically most suitably value of the coefficient 'a.' Several cases will be seen in the table where no possible positive value of the coefficient would make the observed change of average temperature derivable from the change in rectal and in surface temperatures. Yet when the method of taking the average is used, these periods prove to have little effect upon the final value for the coefficient.

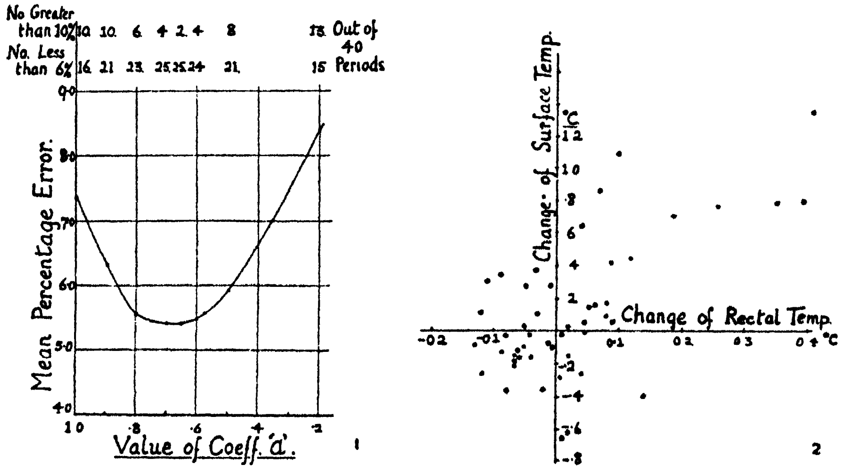
TABLE 3

DATE 1934	THERMAL CAPACITY OF SUBJECT. CALS./°C.	ΔH = INDIRECT HEAT—DIRECT HEAT	ΔT_A	ΔT_R	ΔT_S	Y	X	ΣY^2	ΣXY	a
4–16	71.9	— 2.79	—.039	—.11	+.30	—.41	—.34	.168	.139	.83
	71.9	— 1.09	—.015	—.03	+.097	—.127	—.112	.184	.153	.83
5–2	46.5	— 1.36	—.030	+.08	+.081	.00	+.051	.184	.153	.83
	46.5	— 3.87	—.085	—.01	—.086	+.076	+.001	.189	.153	.81
	46.5	+ 2.94	+.063	+.09	+.411	—.321	—.348	.292	.265	.91
	46.5	— 2.10	—.045	—.005	—.292	+.287	+.247	.375	.336	.90
5–7	46.5	— 3.78	—.081	+.14	—.413	+.553	+.332	.678	.519	.77
	46.5	— 4.84	—.104	—.01	+.268	—.272	—.372	.750	.622	.83
	46.5	+ 13.00	+.280	+.09	+.045	+.045	+.235	.752	.638	.85
	46.5	— 3.04	—.065	—.13	—.093	—.037	+.028	.753	.637	.85
5–11	51.5	— 6.79	—.132	—.06	—.172	+.112	+.040	.766	.641	.84
	51.5	— 5.45	—.106	—.04	—.180	+.140	+.074	.786	.651	.83
	51.5	+ 3.58	+.070	—.03	+.380	—.410	—.310	.954	.778	.82
	51.5	+ 0.52	+.010	+.06	+.149	—.089	—.139	.962	.790	.82
5–16	64.8	—10.99	—.170	—.08	—.31	+.23	+.14	1.015	.822	.81
	64.8	+11.96	+.184	+.04	+.65	—.61	—.466	1.387	1.106	.80
	64.8	+ 6.43	+.099	—.04	—.03	—.01	+.13	1.387	1.105	.80
5–23	51.2	—10.65	—.242	—.08	—.370	+.290	+.128	1.471	1.142	.78
	51.2	— 5.73	—.112	—.115	—.257	+.142	+.145	1.491	1.162	.78
	51.2	— 9.50	—.185	—.06	—.130	+.070	—.055	1.496	1.158	.78
	51.2	+ 0.76	+.015	—.05	+.025	—.075	—.040	1.502	1.161	.77
	51.2	— 7.12	—.139	+.02	—.164	+.144	+.025	1.523	1.165	.77
6–12	60.2	— 6.93	—.115	+.04	—.272	+.232	+.157	1.577	1.201	.76
	60.2	+ 3.45	+.057	+.01	+.004	+.006	+.053	1.577	1.201	.76
	60.2	— 3.83	—.064	+.02	+.020	+.000	—.084	1.577	1.201	.76
5–18	45.2	—12.15	—.269	—.06	—.221	+.161	—.048	1.603	1.193	.75
	45.2	—13.00	—.288	+.02	—.623	+.643	+.335	2.015	1.408	.70
	45.2	— 0.58	—.013	—.05	+.267	—.317	—.280	2.115	1.497	.71
	45.2	+ 2.98	+.066	+.02	—.245	+.265	+.311	2.185	1.579	.72
6–15	65.3	— 5.41	—.083	—.01	—.083	+.073	.000	2.190	1.579	.72
	65.3	+ 3.74	+.057	+.08	+.163	—.083	—.106	2.197	1.588	.72
	65.3	1.76	—.027	+.055	+.139	—.084	—.166	2.204	1.601	.73
6–21	63.0	—14.90	—.237	—.065	—.159	+.094	—.078	2.212	1.593	.72
	63.0	— 7.09	—.113	—.005	—.105	+.100	—.108	2.222	1.582	.71
	63.0	+ 0.90	+.014	+.045	—.009	+.054	+.023	2.225	1.583	.72
	63.0	— 1.79	—.028	+.075	+.004	+.071	—.032	2.230	1.581	.71
6–28	55.8	— 5.41	—.097	—.085	—.138	+.053	+.041	2.233	1.583	.71
	55.8	— 5.32	—.096	+.045	—.046	+.091	—.050	2.241	1.575	.70
	55.8	+ 2.17	+.039	+.090	—.065	+.155	+.104	2.265	1.591	.70
	55.8	— 6.30	—.118	+.005	—.196	+.201	+.078	2.306	1.607	.70

It remains to make use of the formula thus derived, namely—

$$\Delta T_A = .70\,\Delta T_R + .30\,\Delta T_S$$

to calculate the change of average temperature from the change in rectal and in surface temperatures, and, using this value to calculate the heat storage or loss by the body, to see how much deviation there is then between direct and indirect heats. Using the rectal temperature alone the average discrepancy in the forty periods was 7.48 per cent, using the surface temperatures also, with 0.70 as the coefficient 'a,' this is reduced to an average discrepancy of 5.47 per cent. The number of periods in which the discrepancy was more than 10 per cent is reduced from 10 to 4 and the number where it is less than 6 per cent increased from 16 to 25. *There is no doubt that by the use of the surface temperatures a great gain in the accuracy of calculating the change in average temperature has been made.*



Figures 1 and 2

In order to see how critical was the choice of the coefficient, the calculation of the average discrepancy has been made using other values for the coefficient, ranging from the value 1.0 (meaning that rectal temperature alone is used) to 0.2. The result is shown graphically in figure 1. Any value of the coefficient between 0.6 and 0.75 gives the improved accuracy. It seems likely that for any one individual the curve would have a steeper fall to a minimum, the choice of the coefficient being more critical. The best value would not be the same, however, for different individuals, for the thin and the obese subject, for instance. The result is that for a large group of subjects the best value is not fixed within very narrow limits. It appears that the value of 0.65 for the coefficient is the best for this group, the gain being even more marked in the reduction of the number of large errors than in the average error. The value deduced theoretically was 0.64.

The curve also shows that the rectal temperature is the best single temperature for the calculation, the use of the surface temperatures alone leading to a very large error.

Correlation between rectal and surface temperatures. In fifteen cases out of the forty in table 3 the changes in rectal and in surface temperatures occur in opposite directions. This suggests that the correlation between the two is not very great, a conclusion which is borne out by the graph, figure 2, which includes the results of fifty-three hour periods. For small changes of temperature there is quite a large probability that the changes may occur in opposite directions, giving points in the diagram lying in the 'N.W.' and 'S.E.' quadrants. For larger changes of temperature there appears to be greater correlation, roughly to the relation—

$$\Delta T_S = 4.\ \Delta T_R$$

The total of all the changes in rectal temperature (without regard to sign) is 4.245°C., while the total of the changes in surface temperature that simultaneously occurred is 16.035°C. The changes in surface temperature were then on the average 3.8 times as great as the changes of rectal temperature. In the equation for the average temperature—

$$\Delta T_A = .65\ \Delta T_R = .35\ \Delta T_S$$

if ΔT_S is 3.8 times as great as ΔT_R, the term in the average arising from the surface temperatures is (since $\frac{.35 \times 3.8}{.65 \times 1.0}$ equals 2.0), more important than the term from the rectal temperature. Hence, *although the rectal temperature has the larger coefficient in the average, the measurement of the surface temperature is of more importance.*

This degree of correlation also suggests that if one temperature alone is to be used, either the rectal or the surface temperatures, better agreement might be obtained by substituting in the equation from the correlation equation: ΔT_S equals $3.8 \Delta T_R$. For instance, using the rectal temperature alone, the equation becomes—

$$\Delta T_A = .65 \Delta T_R + .35 \times 3.8 \Delta T_R = 1.9 \Delta T_R.$$

instead of the usual employed relation, ΔT_A equals ΔT_R. For the whole group, this gives no better average error than using the rectal temperature itself, due to the lack of correlation when the changes are small. The device would obviously be of use only if the rectal temperature changes by more than 0.2°C., no case of which is available for which the direct and indirect heat is simultaneously known. If the surface temperature alone were used the formula would be—

$$\Delta T_A = \frac{.65}{3.8} \Delta T_S + .35 \Delta T_S = .61 \Delta T_S.$$

The errors have been calculated using $.6 \times \Delta T_S$ alone. The resultant average error is 6.72 per cent, with eleven periods having the error greater than 10 per cent and twenty-two with errors less than 6 per cent. This is as good as, if not better than, the result of using the rectal temperature alone. This might conceivably not be true for a larger group of periods, but it is evident that the surface temperature changes may be made to give as good an indication of the changes in average body temperature as the rectal temperature changes.

The average temperature of the body tissues. Having found the value of the coefficient 'a,' the same figure can be used to find the absolute value of the true average temperature of the whole body. Taking a case of a subject in basal condition, with the following temperatures:

Rectal temperature,	$T_R = 36.95$°C.
Average surface temperature on trunk,	$T_1 = 35.05$°C.
on lower leg,	$T_2 = 29.60$°C.
on lower arm,	$T_3 = 28.40$°C.

we find the average surface temperature T_S equals 31.66°C. and the average temperature for the whole body, T_A, equals 35.10°C. In this case the average for the whole body is 1.85°C. below the rectal temperature. This must obviously be taken into consideration when the relation between body temperature and metabolism is being studied.

It has been pointed out (Nasset, '32) that in the curve given by DuBois ('21) for the increase of the metabolism of febrile patients with rise of rectal temperature between 37° and 40°C., the quantity Q_{10}, calculated by the well-known formula—

$$\log Q_{10} = \frac{\log K_1 - \log K_2}{t_1 - t_2} \times 10.$$

is greater for the smaller rises of temperature, say 37° to 38°, than for the larger range, say 37° to 40°. It has been shown by the author (Burton, '34 b) and by many others that in fever the surface temperatures rise a great deal more than the rectal temperature, and, indeed, reach values very close to that temperature. This is the consequence of the increased general and peripheral circulation. At the higher rectal temperatures, then, the average temperature for the whole body, which was from 1 to 2° below the rectal temperature in the basal, afebrile, condition, is now more closely equal to the rectal temperature. The true rise of average temperature is greater than the rise of rectal temperature, so that the Q_{10} calculated from rectal temperature alone is greater than the true Q_{10}. It is obvious that the error will be of greater magnitude for the smaller changes of rectal temperature, since the surface temperature soon approximates to and never exceeds the rectal temperature. It may well be that measurements of surface temperature in fever and the use of the formula deduced for the average temperature would show that the metabolism followed the Arrhenius equation more closely than has appeared.

Measurement of the rectal temperature alone is still less adequate in experiments where the body temperature is raised by high frequency currents, by hot water or hot air baths, for here the surface temperature may actually rise above the rectal temperature. A series of experiments was made in this laboratory in which it was attempted to raise the body temperature of normal subjects in basal condition by high frequency currents, applied by electrodes to the sole of each foot. Skin temperatures were simultaneously taken. It was not found possible to raise the rectal temperature by more than 0.7 or 0.8°C. before copious sweating and discomfort of the subject intervened. Before this occurred, however, the temperature of the surface of the body rose by vastly greater amounts, that of the leg becoming in some cases greater than rectal temperature and that of the trunk and arms approaching it. The metabolism was taken in ten minute periods by the Tissot-Haldane method. An example is given in table 4, and shown graphically in figure 3. In periods 1 and 2 the body temperature was rising, in 3 and 4, the current had been turned off and sweating had cooled the surface greatly, though

TABLE 4

Diathermy experiment February 27, 1934

NO.	RECTAL TEMPERATURE, °C.	SURFACE TEMPERATURE °C.				AVERAGE BODY TEMPERATURE, °C.	METABOLISM, CALS./HR.
		Trunk	Lower leg	Lower arm	Average		
1	36.75	34.86	32.68	32.80	33.79	35.71	63.03
2	37.32	37.32	33.63	36.92	35.52	36.69	67.48
3	37.49	33.58	37.48	33.12	34.92	36.59	67.42
4	37.20	32.63	33.63	32.18	32.93	35.71	59.65

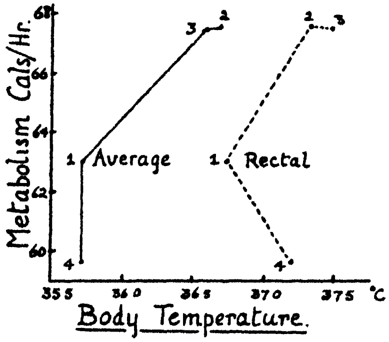


Figure 3

the rectal temperature had fallen only slightly. It will be seen that the metabolism shows little correlation with the rectal temperature, but much more with the calculated average temperature. How much the Q_{10} may be modified is shown by the fact that for points 1 and 2, using rectal temperature alone, Q_{10} is 3.3; using the average temperature, its value is 2.0. It is probable that many of the contradictory results in this field are due to the inadequacy of the rectal temperature to represent the true average temperature of the active tissues.

The specific heat of the human body. In the calculations, the value 0.83 for the average specific heat of the body has been assumed. This figure is the result of averaging the specific heats that have been found, in vitro, for various tissues, weights being given in the average according to the amount of such tissues in the body. The specific heats of tissue vary from 0.70 for fat to nearly unity for blood. A list is given with references, by Pembrey (1898). The specific heat has never been determined experimentally for a whole living man. It must obviously differ in different individuals according to the amount of subcutaneous fat. It is evident that no determination can be made until the calculation of the true average temperature can be made with certainty, and for this we have assumed the value of the specific heat. Making no assumption, but leaving it as 's,' we have—

$$\Delta T_A = \frac{\Delta H}{W.s}$$

and

$$\frac{\Delta H}{W} \times \frac{1}{s} = a \Delta T_R + (1 - a) \Delta T_S.$$

Given a large series of data of simultaneous values of the temperature changes ΔT_R, ΔT_S and $\frac{\Delta H}{W}$, it is possible by the methods of statistics to find the best values not only of the coefficient 'a,' but of the specific heat 's' also. The problem is geometrically that of drawing the best plane through a set of points in three dimensions, as the problems ordinarily encountered are of drawing the best line through a set of points in two dimensions. The computation is laborious and the forty periods here available are not sufficient to make it worth while. An alternative method is the well known one of 'successive approximation.' Having assumed the value of one of the unknowns, as we did that of the specific heat 's,' it is used to find the best value of the other unknown, in this case of the coefficient 'a.' The process is then reversed; using the value of 'a' then determined, the best value of 's' is found. If this value differs greatly from the value originally assumed, the process has to be repeated until an unchanging result is reached. Using the value 0.65 for the coefficient 'a,' the calculation of the errors has been made using values of the specific heat from 0.6 to 1.0. The perhaps surprising result is that for this group of experiments, the choice of the value of the specific heat used in the calculations is not at all critical. The figures for the average errors are:

$s = 1.0$, 5.87 per cent, $s = 0.90$, 5.55 per cent,
$s = 0.83$, 5.52 per cent, $s = 0.70$, 5.56 per cent and
$s = 0.60$, 5.59 per cent.

The figure 0.83 gives only slightly better results than the other values within quite wide limits. From the data it is not possible to determine the average value of the specific heat, except that it lies within the wide limits of 0.7 to 0.9. Rough though this determination is, it is yet the first experimental determination of the specific heat of the living human body. It is evident that, without the measurement and use of surface temperatures, no such determination even of this degree of accuracy, would be possible.

SUMMARY

The temperature of the surface of the human body is 4° or 5° lower than the interior temperature, which is not reached until a depth of several centimeters. A calculation is made showing that about 50 per cent of the body is within an inch from the surface. Account must be taken of this cooler peripheral tissue in calculating the average temperature of the body, especially for the agreement of direct and indirect calorimetry. A theoretical estimate is made of how the rectal

and surface temperatures should be combined to give the true average.

From the results of forty hour periods with the respiration calorimeter upon a group of subjects in basal and absorptive condition, the formula giving the least average discrepancy between direct and indirect heats is found experimentally. It agrees closely with that deduced theoretically, and is: average temperature equals 0.65 × rectal temperature plus 0.35 × average surface temperature. The latter temperature is found from measurements over the trunk, the lower leg and the forearm. The average error is reduced from 7½ per cent using rectal temperature alone to 5½ per cent using the formula.

The changes occurring in surface temperature are on the average three or four times as great as those of the rectal temperature, and are poorly correlated with the latter when the changes are small. The measurement of surface temperatures is equally if not more important than the rectal in the calculation of the average. By using 0.6 times the change in surface temperature alone, the results in this group are slightly better than using rectal temperature alone.

The average temperature from the body is from 1 to 2°C. lower than the rectal temperature in the basal state. In fever the two may be practically equal. Some discrepancies in the calculation of the Q_{10} for metabolism with temperature are explained, and the formula is applied to some experiments where subjects were heated by high frequency currents.

The calculation of the average specific heat of the living human body is made possible for the first time, but the data can show only that it lies between 0.7 and 0.9.

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HUMAN CALORIMETRY

III. TEMPERATURE DISTRIBUTION, BLOOD FLOW AND HEAT STORAGE IN THE BODY IN BASAL CONDITION AND AFTER

ALAN C. BURTON AND JOHN R. MURLIN
Department of Vital Economics, University of Rochester, Rochester, N. Y.

FOUR FIGURES

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Interest in the subject of this research was stimulated by the preliminary report in 1933 by Essex and co-workers ('33) of studies on the blood flow to the liver and to the limbs of dogs after ingestion of food. A paper describing later work of the same authors has recently been published (Herrick et al., '34). It had generally been supposed that during digestion of food, blood was diverted from the somatic tissues to supply the viscera. As these authors state, this belief "has been so general as to give it the force of demonstrated fact." Contrary to expectation, their experiments show that there is a prolonged general increase in blood flow, measured in femoral, carotid, and mesenteric arteries and jugular vein, after ingestion of solid or liquid food.

It seemed of importance that this phenomenon should be studied in man. The method used on dogs, that of the 'thermostromuhr' of Rein ('28) with local anaesthetics, is obviously inapplicable to human subjects. Information as to blood flow must be obtained from observations of the changes in skin temperature. By the use of the 'thermal circulation index' introduced by one of us (Burton, '34 b), measurements of skin temperature may be made to yield reliable information as to the blood flow to the limbs. A study has therefore been

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[1] This work has been aided by a grant from the Rockefeller Foundation.

made of the changes occurring in the rectal and surface temperatures of human subjects in basal condition and after ingestion of food. The measurements have been made simultaneously with calorimetry periods in the respiration calorimeter (described in paper I of this series, Murlin and Burton, '35) so that at the same time the heat production, heat elimination and their difference, the heat storage or loss by the body in changing its average temperature are available. The experiments furnish information in answer to the question of what happens to the extra heat produced in the body by the specific dynamic action of food.

Experimental material. In all, ten adult subjects were used, two being female. Their weights ranged from 51.2 to 85.5 kg., and their heights from 159 to 188 cm., so that they form a diversified average group. The routine of experiment was the same in the majority of the cases, those in which it differed being described later. Some experiments also were made with the subject not in the calorimeter.

The subjects came to the laboratory in basal condition, having had no food since the previous evening. They lay in the bed of the calorimeter while the various thermometers, rectal thermometer, and skin temperature thermometers on the abdomen, on the lower leg and a forearm, were placed in position. The construction and use of these thermometers has already been described, in the first paper of this series (Murlin and Burton, '35) and in detail in an earlier paper (Burton, '34 a). Over them light clothing was worn, of an amount that had been found in previous experiments to make the subject 'comfortable' at the temperature of the chamber (about 23°C.). In the case of the male subjects, this was usually shirt and trousers, covered by a laboratory coat. The chamber having been closed, air and water were circulated in the systems for about an hour in a preliminary period in which thermal and gaseous equilibrium was established. Two calorimetry periods of an hour each followed. The subject then emerged and ate the meal, light exercise being found desirable for the relaxation of the subject making possible the 3 further hours in the calorimeter. About 20 minutes after the meal, the subject reentered the chamber and a preliminary period was followed by two more calorimetry periods, starting an hour after the meal was finished. Two periods in the basal state, and two, the second and third hours after the meal, were thus obtained. The measurements of body temperature, taken every 5 minutes throughout, extend over longer periods, nearly 3 hours in each case. In one experiment the subject remained an extra hour, so that three periods were obtained after the meal, with temperatures followed for 4 hours.

The normal variation between individuals, as well as the errors of calorimetry, render any conclusion based on an individual experiment of little value. Accordingly, in the results that follow, the average of nine similar experiments is given the most weight in the conclusions. Lack of space forbids the listing of the details of all the experiments.

Nature of meals taken. In five experiments of the first group the meal was mainly of carbohydrate, in the other four almost exclusively of protein. The carbohydrate consisted partly of starchy foods like Cream of Wheat, cornstarch, and bread, partly of sugars, cane and Karo syrup, partly of fruits, such as peaches and orange or grape fruit juice. The carbohydrate equivalent of the amount ingested was from 180 to 320 gm. The protein meals were of lean chopped beef, in amounts from 200 to 434 gm. The study is a preliminary one of the effect of food, in general, on a number of subjects. For accurate conclusions upon the comparison of the effects of particular foods, a number of experiments upon the same individual would be necessary with more carefully controlled quantities ingested.

Heat storage. If the heat produced in the body, given by the 'indirect heat' calculated from the oxygen consumption, exceeds the heat eliminated in the same period, the 'direct heat' measured by the calorimeter, heat has been stored by the body, its average temperature rising. Thus in what follows, the quantity called 'heat storage' is defined as the indirect heat minus the direct heat in the period. Negative values of the heat storage, of course, indicate that heat elimination exceeds heat production for the period and the average body temperature has fallen. Alcohol checks with the respiration calorimeter show that it measures the quantity indirect minus direct heat with an average error in hour periods of about 3 per cent. The systematic error is not more than 0.17 per cent, so that the averages of heat storage in a series of experiments may be relied upon.

TABLE 1

Heat storage as percentage of heat produced

DATE 1934	SUBJECT	BASAL		AFTER MEAL				KIND OF MEAL	PER CENT INCREASE IN HEAT PRODUCTION[2]
		1st hour	2nd hour	1st hour	2nd hour	3rd hour	4th hour		
5-2	L.H	— 2.2	— 5.9	(+ 53)[1]	+ 4.1	— 2.6		Meat	20
5-7	L.H	— 5.8	— 7.7	(+ 32)	+ 14.4	— 3.7		C H	24
5-11	J.Ha	— 11.8	— 9.3	(+ 39)	+ 4.9	+ 0.7		C H	24
5-16	G.E	— 12.7	— 2.1	(+ 25)	+ 12.4	+ 7.0		C H	24
5-18	G.Mc	— 21.8	— 23.2	(+ 41)	— 0.8	+ 4.0		C H	31
5-23	K.B	— 21.4	— 11.3	(+ 29)	— 16.9	+ 0.1	— 11.8	Meat	21
6-12	W.		— 10.4	(+ 37)	+ 3.8	— 4.9		C H	16
6-21	J.Hu	— 21.4	— 11.3	(+ 21)	+ 1.2	— 2.1		Meat	22
6-28	R.R	— 7.8	— 7.7	(+ 44)	+ 2.5	— 9.0		Meat	26
	Average	— 13.1	— 9.6	(+ 36)	+ 2.8	— 1.2	(— 11.8)		23

[1] Percentage of basal heat production stored.

[2] Average percentage increase of second and third hours over basal.

Table 1 gives the results of nine experiments. The heat storage, defined above, is given as a percentage of the heat produced by the subject in that hour.

In the basal condition the heat storage in the first hour is invariably negative, the average body temperature evidently falling. The average loss is 13.1 per cent of the heat produced. A review of previous periods in the basal condition (in experiments not related to this study) gives approximately the same figure (— 13.5 per cent). It does not appear that this loss of heat is due to the temperature of the calorimeter chamber (on the average 23.3°C.) being abnormally low, as the loss is not correlated with the environmental temperature at all well. It has been repeatedly observed in this laboratory and elsewhere that the rectal and surface temperatures in basal condition continue to fall steadily for as long as 2 hours when the subject lies down and relaxes completely, even in an environment with a temperature as high as 27°C. The net loss of heat must be considered as the effect of immobilization of the subject, which produces a large decrease in the heat production.

The fact that the average net heat loss in the second of the basal periods is lower than that of the first hour, —9.6 per cent compared to —13.1 per cent, is also confirmed in review of previous experiments in the basal condition. In some cases there was an actual storage of heat or a negligible heat loss in the second of two basal periods, in the first of which there had been considerable net loss. This decrease cannot be attributed to any difference in the temperature of the chamber, which was only 0.07°C. higher on the average. Heat production in the two periods was approximately the same, the average for the second period being 1.3 per cent higher than for the first. The bearing of these results on heat loss on the question of heat regulation of the body is discussed later. The magnitude of the discrepancy between heat production and heat elimination in the completely relaxed basal subject emphasizes the need for accurate estimation of heat storage from changes in body temperature if the two are to be brought into agreement (paper II of this series).

For the hour in which the subject was out of the calorimeter and eating the meal, the heat storage can be estimated only from the change that was observed in the body temperatures. It has been calculated using the formula developed in paper II (Burton, '35) for each experiment and is given in the fifth column of table 1 (in brackets). Since the heat production in this period is not known, it is expressed as a percentage of the basal heat production. If expressed as a percentage of the actual heat production in that period it would, of course, be less. There is no doubt that in this period there is a large positive storage of heat. How much is due to the exercise of the subject and how much to the food cannot be stated.

In the succeeding hour, the second hour after the ingestion of food, the heat production tends still to exceed the heat elimination, so that the heat storage is positive. In the next hour the storage becomes slightly negative in the average, the heat production having passed its peak while the heat elimination is still increasing. It is evident that the reactions are slower in some subjects than in others, the positive heat storage occurring in the third hour instead of the second. Presumably this is due to differences in the rates of digestion and absorption. In the single experiment where a third hour is available the body had begun to lose heat once more to a considerable amount.

In only one case can the effects of carbohydrate and protein be compared in the same subject. Here the reaction was evidently more rapid and of a higher peak value with the carbohydrate meal.

The increase in the average rate of heat production in the second and third hours after the meal, over that in the basal periods, is given as a percentage of the latter in the last column. It expresses the usual 'specific dynamic action' of food, in the manner originally used by Rubner and adopted by Lusk.

The observed sequence of heat storage and loss is the consequence of the fact that the extra heat elimination after the meal lags behind the extra heat production, so that there is a temporary rise in body temperature until the increased heat elimination finally compensates. We are studying here the fluctuations and lag of the human thermostat as it meets fluctuations in heat production. In the sections that follow the mechanism of the increased heat elimination is examined more closely.

Water loss. In table 2 the results for the heat lost by evaporation for the same group of experiments are listed. The average percentage of the heat produced that is lost by evaporation is the same for the two basal periods, namely, 17.8 per cent. This figure is considerably less than that usually found, namely, 24 per cent of the heat loss (Soderstrom and DuBois, '17) although the environmental temperature was in the same range. The same authors found an insignificant decrease to 23 per cent after ingestion of glucose or of protein. In our experiments the decrease in the percentage is quite marked in the second hour after the meal. In the third hour sweating occurred in some of the experiments (shown by the changes in skin temperature and noted by the subject) and the percentage is only slightly less than the basal. The decrease in percentage, expressed in terms of the heat produced, is not an indication that the evaporation loss decreased after the meal in absolute value. On the contrary, there was a slight increase, as shown by the table. The apparent decrease is due to the fact that the heat lost by evaporation does not increase as rapidly as does the heat production. When expressed as a percentage of the heat lost in the period, the figures are remarkably constant, 15.8 per cent, 16.5 per cent, 16.5 per cent, 16.5 per cent, respectively. This suggests that in this respect the body is losing heat as a purely physical system.

TABLE 2

Percentage of heat produced lost by evaporation and as Cals./sq.m./hr. lost by evaporation

DATE 1934	SUBJECT	BASAL				AFTER MEAL					
		1st hour		2nd hour		2nd hour		3rd hour		4th hour	
		Per cent	Cals.	Per cent	Cals.	Per cent	Cals.	Per cent	Cals.	Per cent	Cals.
5–21	L.H	20.0	7.8	20.1	8.3	15.9	7.1	17.5	8.7		
5–7	L.H	16.6	6.6	17.9	7.0	15.2	8.4	16.6	8.4		
5–11	J.Ha	15.1	5.0	16.1	5.5	13.1	5.6	15.4	6.2		
5–16	O.E.	12.9	4.6	12.3	4.8	10.4	4.9	13.1	5.8		
5–18	G.Mc.	18.1	6.4	17.4	6.2	15.8	7.2	18.1	8.5		
5–23	K.B.	17.5	5.4	17.5	5.5	17.4	6.0	16.3	6.5	18.3	6.8
6–12	W.			17.0	6.9	17.7	8.9	20.3[1]	9.0[1]		
6–21	J.Hu	21.0	7.8	21.4	8.1	18.0	8.4	17.7	8.2		
6–28	R.R	21.9	8.3	22.2	8.6	19.8	9.8	22.2[1]	10.4[1]		
Average		17.8	6.5	17.8	6.9	15.9	7.4	17.5	7.9		
Av. percentage of heat elimination		15.8		16.5		16.5		16.5			

[1] Sweating occurred.

Radiation and convection. Table 3 shows that the burden of the elimination of the extra heat is borne by the increased radiation and convection from the body. There is no doubt about the considerable increase in the hours following the food. Comparison of the averages at the foot of the table for radiation and for heat production brings out the fact, mentioned earlier, that the heat elimination lags behind the heat production. The latter has passed its peak by the third hour, while the former is still rising.

TABLE 3

Radiation and convection in Cals./sq.m./hr.

DATE 1934	SUBJECT	BASAL		AFTER MEAL		
		1st hour	2nd hour	2nd hour	3rd hour	4th hour
5-2	L.H.	31.8	34.6	36.0	42.3	
5-7	L.H.	35.5	35.4	40.1	44.4	
5-11	J.Ha	32.2	31.6	34.9	33.8	
5-16	G.E.	35.5	34.8	36.7	35.7	
5-18	G.Mc.	36.6	37.6	38.9	40.9	
5-23	K.B.	31.8	29.4	34.6	33.0	34.5
6-12	W.		38.0	39.4	37.8	
6-21	J.Hu	37.1	33.4	37.8	37.1	
6-28	R.R.	31.7	32.0	37.4	39.6	
Average		34.5	34.1	37.3	38.3	
Average heat produced		36.5	37.4	46.3	45.8	

From physical principles it is clear that the increase in radiation and convection could be brought about only by an increase in the temperature of the surface of the body, the heat originating, of course, from metabolism.

Temperature changes. The skin temperature thermometers measure directly the average temperature over portions of the surface of the body, which are in area about 930 sq.cm. for the trunk, 600 sq.cm. for the lower leg, and 360 sq.cm. for the forearm. Care was taken that the conditions of clothing worn over these thermometers should be identical throughout the experiment, so that the observed changes might be of physiological significance. The temperatures showed considerable consistency, the fluctuations, as distinguished from systematic trends, rarely exceeding 0.1°C. from one observation to the next, 5 minutes later. The occurrence of sweating could be seen at once in the sudden drop of temperature and the subsequently increased fluctuations. Muscular movements, as those of the arm in operating the signaling device in the chamber, raised the temperature momentarily. In the averages taken over the whole period, a change of skin temperature of more than 0.1°C. can be considered significant.

In the basal period the rectal temperature continued to fall for almost the whole of the first 2 hours that the subject was lying down, remaining, on the average, constant or even rising in the third hour. The surface temperatures, however, falling with the rectal at about the same rate for the first hour, fell much more rapidly in the second and third hours. This is brought out by the averages for all the experiments in table 4. (The average surface temperature is calculated from 0.5 X trunk plus 0.36 X leg plus 0.14 X arm temperatures, a formula weighting the temperatures according to the mass of tissue beneath them.) The fall is most marked on the leg, least on the arm. The total fall of surface temperature is much greater than that of rectal temperature. In one female subject, the temperature on the surface of the leg fell a total of 3.4°C. in the 3 hours, from 31.5° to 28.1°C., while the rectal fell only 0.25°C.

TABLE 4

Average temperature changes in °C.

PLACE	BASAL		AFTER MEAL		
	1st hour	2nd hour	1st hour	2nd hour	3rd hour
Rectal	—0.09	+0.02	+0.26	+0.02	0.00
On trunk	—0.06	—0.15	+0.78	+0.25	—0.12
On lower leg	—0.23	—0.39	+0.93	+0.09	0.00
On forearm	—0.01	—0.21	+0.80	+0.08	—0.16
Average surface	—0.08	—0.25	+0.79	+0.18	—0.08

These changes of temperature give evidence of the operation of the heat regulating mechanism of the body against loss of temperature. In the first hour the body cools as a purely physical system, the surface falling an amount approximately equal to the fall of interior temperature. In the following hours, however, the surface falls a great deal more than the interior, indicating a decrease in the heat conductivity of the peripheral tissues, due to a vasoconstriction and decreased blood flow. That this physiological adjustment is effective is proved by the cessation of the fall of rectal temperature and by the decrease in the net heat loss (table 1).

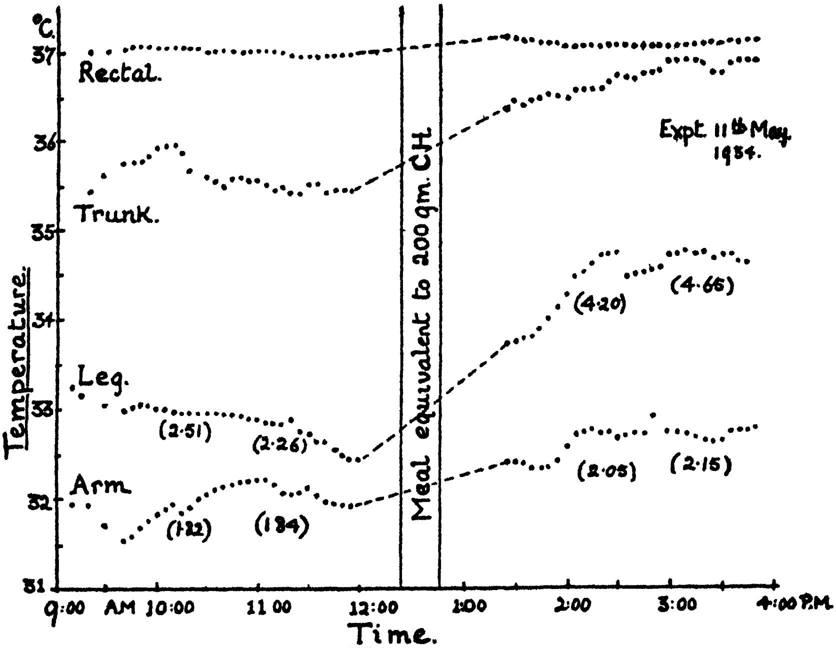

Figure 1

In the hour immediately following the meal the rectal temperature is the first to rise, remaining on the average steady in the next 2 hours. The surface temperatures show a very much greater increase, which continues in the second hour, falling finally in the third. In figure 1 the records of temperature of one experiment are plotted to show the time relations of the changes. Without exception in the experiments, the surface temperatures were maintained at a much higher level after the meal, even though in some cases the rectal temperature had fallen. The average levels of the surface temperatures during the hour periods are given in table 5. The rise of temperature is usually the greatest for the surface of the lower leg, least for the forearm (as explained later, however, this does not mean that the change in blood flow is greatest in the legs).

Effects of exercise. It is obvious that in the periods following the meal, the effects of immobilization of the subject are superimposed upon those due to the ingestion of the food. Since, however, exercise preceded the basal periods just as it did the periods following the meal, a comparison of the changes of temperature in corresponding periods tends to eliminate this factor. In order that the time relations of the temperature changes due to the food might be examined unaccompanied by the effects of exercise, an experiment was made in which the subject lay in the calorimeter in basal condition for about 2 hours, the second of which was a calorimetry period. He then, at a signal, ate a meat meal while lying there, making as few muscular movements as possible, and two calorimetry periods of an hour each followed.

TABLE 5

Average level of temperatures in °C.

PLACE	BASAL		AFTER MEAL	
	1st hour	2nd hour	1st hour	2nd hour
Rectal	36.72	36.68	36.92	36.96
Trunk	34.52	34.36	35.13	35.07
Lower leg	32.16	31.78	32.60	32.84
Lower arm	31.32	31.09	31.75	31.65
Calorimeter chamber	23.30	23.37	23.40	23.59

There was an immediate small rise of rectal temperature of about 0.08°C. and of about 0.4°C. in the temperature on the trunk after the meal was finished, but this proved temporary only. No simultaneous rise was recorded in the temperatures on the leg or arm. About 1½ hours later, however, a significant and rapid rise in surface temperatures was evident. As the subject had been in the calorimeter for 4 hours, the rise could be followed for some 20 minutes only. The plausible explanation of this delayed rise is that in this experiment the emptying of the stomach and absorption had been delayed, and that as Herrick and co-workers ('34) found in their experiments on dogs, the increased blood flow does not occur unless there is absorption. The value of heat production in the 3 hours bears out this conclusion. It was 78.9 Cals./hr. in the first, basal hour, 89.3 in the hour during which there was exercise, and 82.8 in the last hour during complete quiescence. The specific dynamic action had only just started when the experiment ended. A second experiment of this character failed, because of the inability of the subject to ingest the meal while in the prone position.

Two experiments were made in which the surface and rectal temperatures were taken on a subject lying on a bed in a cloth covered cubicle, the walls of which contained resistance wire thermometers. No metabolism studies were made in order that disturbing effects due to lack of relaxation of the subject might be eliminated. The meal, 200 gm. of lean chopped beef, was eaten by the subject still on the bed, but with the head and shoulders propped up. It is thought that these experiments give a true picture of the effects of food ingestion, with the least complication due to muscular exercise. One of them is illustrated in figure 2. A control experiment with similar conditions, but no ingestion of food is shown in the same charts. Comparison enables the time relations of the temperature changes to be estimated fairly accurately. There was an immediate rise in rectal temperature, and a very rapid rise of the temperature of the leg, even though this had not been moved at all. The apparent cooling of the arm after the meal is seen to be the normal fall in the basal condition until the rise began some 30 minutes after the meal was finished. The maximum was reached at about 1½ hours after ingestion.

The data for another experiment, in which the metabolism was taken in ten minute periods every hour by the Tissot-Haldane method, are given in table 6. The periods were of

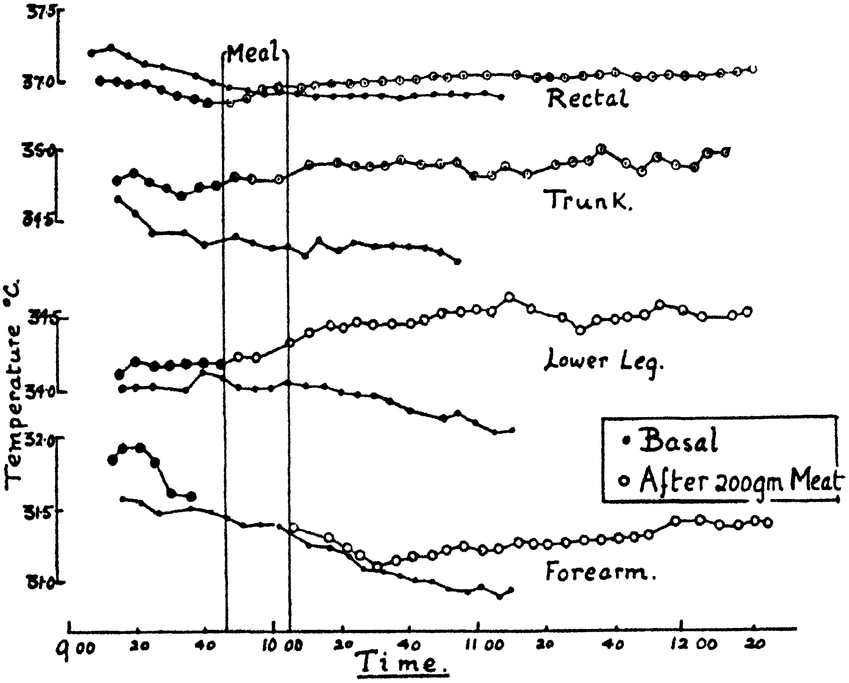

Figure 2

TABLE 6

Period	I	Meal	II	III	IV
R.Q.	.82		.80	.80	.80
Metabolism Cals./hr.	65.5		73.0	79.3	82.8
Increase over period I			11.4%	21.2%	26.4%
Average rectal temperature, °C.	36.97		37.08	37.09	37.13
Increase over period I, °C.			.11	.12	.15
Average temperature on trunk, °C.	34.77		34.93	35.40	35.44
Increase over period I, °C.			.16	.63	.67
Average temperature on leg, °C.	32.55		33.13	33.74	33.38
Increase over period I, °C.			.58	1.19	.83
Circulation index trunk	4.02 (100)		4.01 (100)	5.43 (135)	5.49 (137)
Circulation index leg	1.50 (100)		1.72 (113)	2.24 (149)	1.92 (128)

1 hour each, and the meal was eaten at the start of the second hour. The results, so far as temperature changes go, are comparable with those shown in the charts. The last lines of the table show the thermal circulation index.

Finally, two experiments were made with the subjects lying unclothed in a room of constant temperature and humidity.[1] The changes in the average temperatures on the abdomen are shown in figure 3. The changes on the leg and forearm followed a similar course. The drop in temperature immediately following the ingestion is due to the fact that the solution was at low temperature (about 15°C.), and corresponds well in amount with that calculated to supply heat sufficient to raise the weight of fluid to body temperature. The difference in the time relations of the reactions is marked, and corresponds with the fact, known from previous experiments, that absorption and diuresis are slower in subject B than in A. There was definite glycosuria in A, but none detectible in B after the sucrose ingestion.

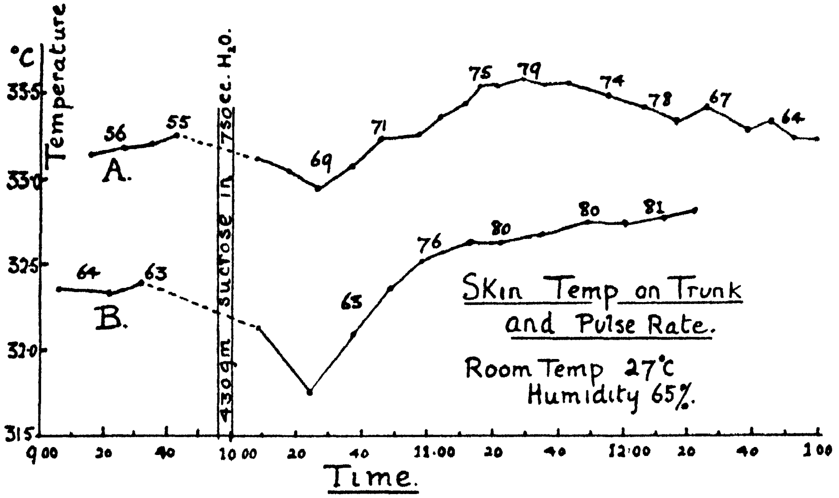

Figure 3

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[1] Coincident studies of insensible loss in short intervals by weighing and of urine excretion were carried on by Mr. G. McClure and Mr. J. Hall, for whose courtesy in using the data we are indebted.

The whole group of experiments leaves no doubt that there is a significant rise of surface temperature of the human body much greater than the coincident rise of rectal temperature, after the ingestion of solid or liquid carbohydrate or protein meals. The failure of Herrick and co-workers (loc. cit.) to find any significant rise of temperature on the forearm of a human subject or the leg of a dog must be attributed to the insensitiveness of their apparatus or lack of control of environmental conditions. It is interesting to note that Williams, Riche and Lusk ('12) recorded that there was a rise in skin temperature of a dog after ingestion of food greatly exceeding the rise of rectal temperature.

Blood circulation. The fact that the changes in surface temperature both in the basal and absorptive periods, exceed the changes in internal temperature, means that there have been changes in circulation of blood, since, in a purely physical system where the properties of heat transfer are constant, the changes in surface temperature must be less than those of the interior. This is expressed quantitatively by the use of the 'thermal circulation index' (Burton, '34 b).

$$\text{T. C. I} = \frac{\text{External drop of temperature}}{\text{Internal drop of temperature}} = \frac{\text{Surface temp.—Room temp.}}{\text{Rectal temp.—surface temp.}} = \frac{C}{C^1}$$

The index compares the coefficient of heat transfer, for the tissues, C^1 with that for the environment, C. An increase in the index for a given surface area is brought about by increased local or general circulation, a decrease by the reverse. Constancy of the index means that the body is behaving as would a purely physical system of constant thermal properties.

A correction to the simple formula is required if the evaporation from the body changes greatly. In the case of the calorimetry experiments, the results of which are listed in tables 2 to 5, this correction may be made with accuracy. We have, for the heat lost by radiation and convection from an area of surface (surface temp. T_s, environmental temp. T_ε)

$$H_R = C\,(T_s - T_\varepsilon).$$

and for the total heat brought up to that area of surface—

$$H = C^a (T_i - T_s)$$

Then, by division—

$$T.\,C.\,I = \frac{C^a}{C} = \frac{T_s - T_e}{T_i - T_s} \times \frac{H}{H_R}$$

The correction factor $\frac{H}{H_R}$ may be obtained from tables 2 and 3, if we assume that for the particular area in question it is equal to the ratio of the total heat eliminated in the period to the total heat lost by radiation and convection in that period.

TABLE 7

Thermal circulation indices

PLACE	BASAL		AFTER MEAL	
	1st hour	2nd hour	1st hour	2nd hour
Trunk	6.66 (100)	5.70 (86)	7.87 (118)	7.32 (110)
Lower arm	2.30 (100)	2.07 (90)	2.55 (111)	2.73 (119)
Forearm	1.76 (100)	1.66 (94)	1.94 (110)	1.83 (104)

The correction in the case of the average temperatures of table 5 proves to be insignificant, the factors for the four periods being 1.18, 1.20, 1.20 and 1.20, respectively. The corrected indices are given in table 7. For comparison they are reduced so that the figure for the first basal period is 100 (in brackets).

The index is significantly greater after the meal than in the corresponding basal periods, by from 10 to 27 per cent. In individual experiments the increases in the indices were often of much greater amount, from 30 to 50 per cent, the averaging of experiments with differing time relations leading to lower figures. The figures for the experiment of table 6 are given at the foot of that table, and indices are also given (in brackets) on the chart (fig. 1). The use of the T. C. I.

shows that though the changes in temperature on the leg are greater, those on the trunk are just as significant as an indication of increased blood flow. The reaction in the arm seems of smaller magnitude. There is no indication whatever of any decrease of blood flow to the peripheral tissues, but very definitely to the contrary, there is a general increase of circulation after ingestion of food.

Pulse rate. In some of the calorimeter experiments and in those illustrated in figure 3, the pulse rate was taken at frequent intervals. In the basal condition there was only a slight tendency for the rate to decrease after the first few minutes, but after the meal there was a marked increase. That this is closely correlated with the increase of surface temperature is shown in figure 3. The increased peripheral blood flow indicated by the rise of T. C. I. need not then be ascribed to any vasomotor shift, but is explained (as in the dog by Herrick and co-workers) by a general increase in blood flow throughout the body. The cooling of the surface in the later basal periods is more likely due to a peripheral vasoconstriction.

CONCLUSIONS

The inferences from the experiments have been discussed under the various headings, but may be illustrated by figure 4, which shows the results of a fictitious experiment deduced from the average values of all the experiments. The chart is self-explanatory. The dotted area of the blocks shows the net storage or loss of heat in the hour periods. If the full line (heat production) is above the dotted line (heat elimination) the storage is positive, and vice versa.

The stimulation of the heart rate and consequent rise of the surface temperature more rapidly than the interior may well be coincident with the rise of heat production (S. D. A). The thermal capacity of the body, however, would make the rise of temperature lag behind the heat production and persist some time after the latter had reached its peak. We have, at present, no evidence that contradicts the assumption that the circulatory and thermal reactions are stimulated by the same factors as the specific dynamic action. Careful comparison of the effects of protein and carbohydrate may later provide such evidence.

The supposed vasomotor shift of blood from the periphery to the viscera during absorption is seen to be contradicted by the facts. Such a shift would lead to a cooling of the body surface instead of a rise, and some other mechanism, such as increased sweating, would have to be called upon to bring about elimination of the extra heat produced and prevent excessive rise of body temperature. The actual mechanism of a general increase of blood flow throughout the body serves the double purpose of supplying the viscera with the additional blood required by absorption and also of eliminating the extra heat produced.

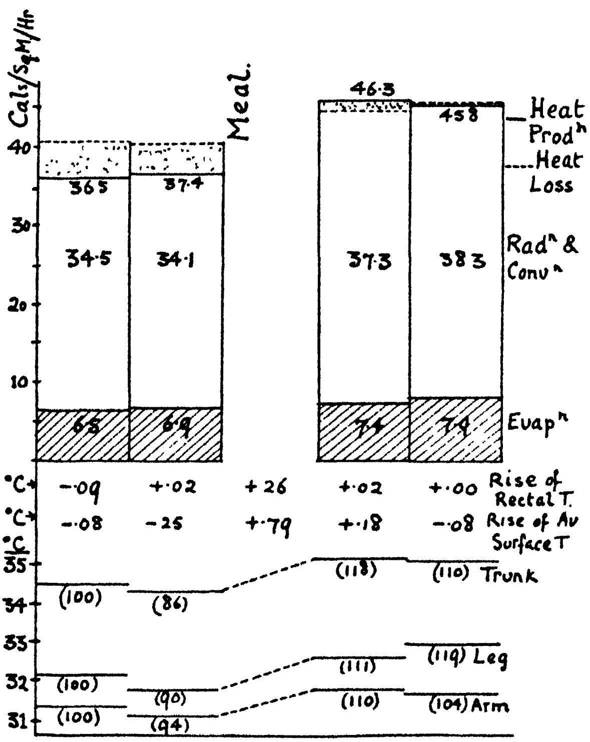

Figure 4

SUMMARY

The heat production, heat elimination, and the rectal and surface temperature changes of ten adult subjects have been studied in the respiration calorimeter, in basal condition and after the ingestion of carbohydrate and protein meals.

On the average, heat elimination exceeds heat production by 13 per cent in the first basal hour and 9 per cent in the second. The body first falls in temperature uniformly; later, the surface falls much more than the rectal temperature, indicating a heat regulating adjustment.

After the meal heat is first stored in the body by general rise of temperature, but later the surface temperature rises much more than the rectal temperature (about 1°C. compared to 0.25°C.), radiation from the body is increased and the extra heat is eliminated. Increased evaporation plays a minor role. The rise of surface temperature is most marked on the leg, but the use of the 'thermal circulation index' shows the increased circulation is general. It commences 20 minutes after the ingestion. The effects of protein and of solid and liquid carbohydrate are similar. They are closely correlated with an increase pulse rate after the meal.

There is no evidence of a withdrawal of blood from the somatic tissues to the visceral, but a definite general increase in circulation, which may well be due to the same factors as cause the specific dynamic action.

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THE PHYTIN PHOSPHORUS OF THE CORN COMPONENT OF A RACHITOGENIC DIET[1]

ROBERT S. HARRIS AND JOHN W. M. BUNKER

Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge

ONE FIGURE

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It has long been assumed that the three dietary factors of importance in the production of experimental rickets in rats are low content of vitamin D, low percentage of phosphorus in respect to the total diet (0.25 per cent or less) (Brown et al., '32), and a low proportion of phosphorus to calcium (not more than 1:4) (Steenbock and Black, '25). That there are other factors of importance and that the classical concepts just stated are in error can now be safely asserted. The early observations of E. Mellanby ('21) on the anti-calcifying properties of oatmeal are not explained by the three primary criteria for rachitogenic diets; recent work of Kon and Booth ('34) indicates an anti-rachitic effect of the saponifiable fraction of butter fat; diets compounded and used in our own laboratory, in work to be reported later, which were devoid of extractable vitamin D, low in total phosphorus, and with Ca:P ratios as exaggerated as 8:1, have failed to induce rickets.

In a previous contribution (Harris and Bunker, '34) we reported variations in the rachitogenic properties of forty samples of corn (maize) compounded into batches of the Steenbock no. 2965 rachitogenic ration (Steenbock and Black, '25), all ingredients other than corn being identical to all lots of diet. Data were supplied to show that these variations were due not to differences in amount of total phosphorus, nor to the Ca: P ratios of the various batches of diet, but were associated with some variations residual in the corns themselves. We suggested that in the corn component there might be linkages of phosphorus with other chemical elements constituting compounds which vary among each other in respect to their assimilability and in respect to their relative proportions in different samples.

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[1] Contribution no. 47 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The establishment of the classical Ca: P = 4: 1 ratio of a rachitogenic diet appears to be the result of determinations of the total calcium and total phosphorus in diets which previously had empirically proved to be rachitogenic. It is no discredit to the original investigators in this field that what appeared to be a causative correlation between calcium-phosphorus ratios and the development of experimental rickets now is perceived to be a relation applying only to certain limited conditions, as for instance, those obtaining in a rachitogenic formula comprising a large proportion (76 per cent) of corn.

If the hypothesis be valid that one or more forms of phosphorus which contribute to the sum of total phosphorus, as usually determined by drastic chemical methods, is of particular metabolic significance to the rat, then we have at least a hypothetical explanation of the failure of the classical total calcium-total phosphorus ratio to support rickets in certain experimental diets. Support of this hypothesis is drawn by us from the papers of Lecoq and Villette ('33) and of Bruce and Callow ('34) in which the addition to diets of phosphorus in various forms (mostly inorganic) has received attention from the point of view of its effect on healing of rickets. If variations in the form of phosphorus administered produce different effects upon the metabolism of healing, there may well be analogous effects upon the metabolism associated with the causation of rickets varying with the type of phosphorus fed. The really significant criteria concerning the phosphorus content of a rachitogenic diet may be restricted to the quantities or proportions of certain types rather than to the total phosphorus values.

The present contribution is based on the first of our studies of the significance of the several fractions of rachitogenic diets which contain phosphorus in one form or another, and deals specifically with the phosphorus of the phytin fraction of corn. Phytin was selected as obviously being quite different in its relation to the total phosphorus of animal and vegetable dietary ingredients, and because we have observed that a rachitogenic diet can be compounded more successfully from corn, for instance, than from casein. Phytin accounts for a large proportion of the total phosphorus of corn; it is absent from casein.

We have estimated the amount of phytin P in each of the forty corns which were the subject of the previous study, using our modification (Harris and Mosher, '34) of the Heubner-Stadler method. The results are given in table 1, column 5. The determinations of the total phosphorus of these corns (column 2) and the animal experiments were carried out at the same time. The quantitations of the phytin phosphorus were made several months later.

The only other source of phosphorus in the diet no. 2965 is wheat gluten, which comprises 20 per cent of the ration. In the diet as fed, the gluten contributed 0.068 per cent total phosphorus and 0.037 per cent phytin phosphorus. As the gluten used in the animal experiments was the same in all cases, the phosphorus fractions contributed by it did not contribute to the variations with which we are concerned.

The purpose of this study was to find whether a correlation exists between the phytin phosphorus content of corn and the degree of rickets it produces when incorporated in the no. 2965 Steenbock ration. To assist visualization of this relationship the total phosphorus and the phytin phosphorus content of the corn in each of the forty rations have been plotted against the degree of rickets each ration produced (fig. 1). There is no apparent correlation.

TABLE 1

CORN NO.	BREED AND SOURCE	1932 ANALYSES			1934 ANALYSES			PER CENT CORN P IN NO. 2965 DIET AS FED [1]	
		Per cent moisture	Per cent total P		Per cent moisture	Per cent phytin P			
			Wet	Dry		Wet	Dry	Total	Phytin
1	Haskins yellow, Mass.	8.4	0.285	0.312	7.4	0.229	0.247	0.218	0.172
2	Golden Glow Dent, Mass.	8.2	0.351	0.382	8.4	0.253	0.276	0.266	0.193
3	Lancaster Sure Crop, N. J.	12.1	0.324	0.368	8.6	0.240	0.262	0.246	0.175
4	Clarage, Ohio	8.4	0.301	0.332	8.8	0.250	0.273	0.232	0.190
5	Lancaster Sure Crop, Del.	14.0	0.289	0.336	10.4	0.135	0.150	0.220	0.098
6	Minn. 13 (Sd. 86), S. Dak.	8.0	0.370	0.400	8.0	0.315	0.342	0.280	0.239
7	Williams Yellow Dent, Mass.	8.3	0.333	0.364	8.1	0.241	0.262	0.254	0.182
8	Minn. 13, Thorpe, N. Dak.	11.5	0.342	0.396	8.2	0.265	0.289	0.266	0.195
9	Duncan Yellow Dent, Mich.	11.4	0.356	0.400	9.0	0.245	0.269	0.269	0.181
10	Not Known, Wis.	9.0	0.326	0.359	9.6	0.247	0.273	0.248	0.189
11	Thatcher's Gold Dent, Ark.	7.9	0.295	0.320	9.3	0.249	0.274	0.224	0.192
12	Not Known, Ill.	13.7	0.293	0.340	8.4	0.230	0.251	0.223	0.164
13	Longfellow-Davis Flint, Mass.	9.3	0.273	0.300	11.5	0.194	0.219	0.207	0.151
14	Not Known, Ill.	12.4	0.314	0.358	9.2	0.279	0.307	0.238	0.204
15	Hulsart Yellow Dent, N. J.	13.3	0.351	0.404	8.9	0.273	0.299	0.266	0.197
16	Kruger, Ill.	12.3	0.326	0.372	8.3	0.190	0.207	0.248	0.138
17	Golden Glow, Mich.	11.6	0.313	0.354	8.2	0.250	0.272	0.238	0.182
18	Yellow Dent seed corn, Utah	12.2	0.376	0.427	8.3	0.293	0.319	0.284	0.213
19	Sure Crop, Penn.	8.0	0.317	0.345	8.1	0.243	0.264	0.242	0.185
20	Hall's Gold Nugget Flint, N. J.	12.7	0.355	0.407	8.3	0.248	0.270	0.270	0.179
21	Polar Dent, Mich.	11.6	0.381	0.432	7.9	0.271	0.294	0.290	0.198
22	St. Paul 13 Dent, Minn.	12.0	0.364	0.414	8.2	0.280	0.305	0.277	0.204
23	MAC Yellow Dent, Mich.	11.8	0.326	0.370	7.9	0.224	0.243	0.248	0.163
24	Reid's Yellow Dent, Neb.	13.8	0.335	0.389	8.7	0.230	0.252	0.255	0.165
25	Golden Queen, Penn.	8.2	0.312	0.340	7.8	0.267	0.290	0.238	0.202
26	Western Plowman, Ill.	12.9	0.306	0.351	8.1	0.223	0.243	0.232	0.161
27	Reid's Yellow Dent, Del.	13.6	0.275	0.318	9.6	0.136	0.150	0.209	0.099
28	Reid's Yellow Dent, N. J.	12.8	0.332	0.381	10.0	0.243	0.270	0.252	0.179
29	Not Known, Kan.	8.4	0.343	0.375	8.0	0.261	0.284	0.261	0.198
30	Wilson Yellow Dent, Fla.	12.2	0.355	0.404	8.5	0.252	0.274	0.270	0.183
31	Somerset Teaming Dent, N. J.	12.9	0.279	0.320	11.8	0.202	0.229	0.212	0.152
32	Cattle, Neb.	14.0	0.372	0.435	9.4	0.245	0.270	0.284	0.176
33	King Philip Hybrid, Calif.	12.4	0.354	0.404	7.9	0.231	0.251	0.269	0.167
34	Pickett Yellow Dent, Mich.	11.7	0.300	0.340	8.7	0.239	0.262	0.228	0.176
35	Reid's White Cap, N. J.	12.0	0.337	0.383	7.4	0.289	0.312	0.256	0.208
36	Mercer Flint, N. Dak.	11.2	0.332	0.374	7.9	0.227	0.247	0.252	0.166
37	Mercer White Cap Yellow Dent, N. J.	12.2	0.300	0.342	11.2	0.251	0.284	0.228	0.189
38	Clement's White Cap, Mich.	12.1	0.300	0.341	8.9	0.223	0.245	0.228	0.163
39	White Flint, R. I.	12.9	0.364	0.418	7.4	0.278	0.300	0.277	0.198
40	Moore's White, Wis.	12.5	0.296	0.338	15.0	0.195	0.229	0.225	0.152
	Average	11.1		0.369	8.9				
	Column number	1	2	3	4	5	6	7	8

[1] On basis of wet weight in 1932.

DISCUSSION

A decade or more ago, E. Mellanby ('21) noted that cereals are rachitogenic (anticalcifying) in spite of their relatively high total phosphorus content. He could not find that the anticalcifying effect is correlated with the fat, carbohydrate, relative or absolute amounts of calcium and phosphorus, nor acid-base relations of their rations. Steenbock et al. ('30) reported more recently that the calcifying property of certain grains, supplemented by calcium carbonate, could be arranged in the descending order, wheat, oats, corn, and that the total phosphorus of these grains is in the reverse order. These findings and others have been discussed by Bruce and Callow ('34) in a report which has appeared while our own data were being analyzed. They advance the view that inositol hexaphosphoric acid (phytic acid) may be the elusive factor in these cereal diets, exerting non-calcifying effect because of

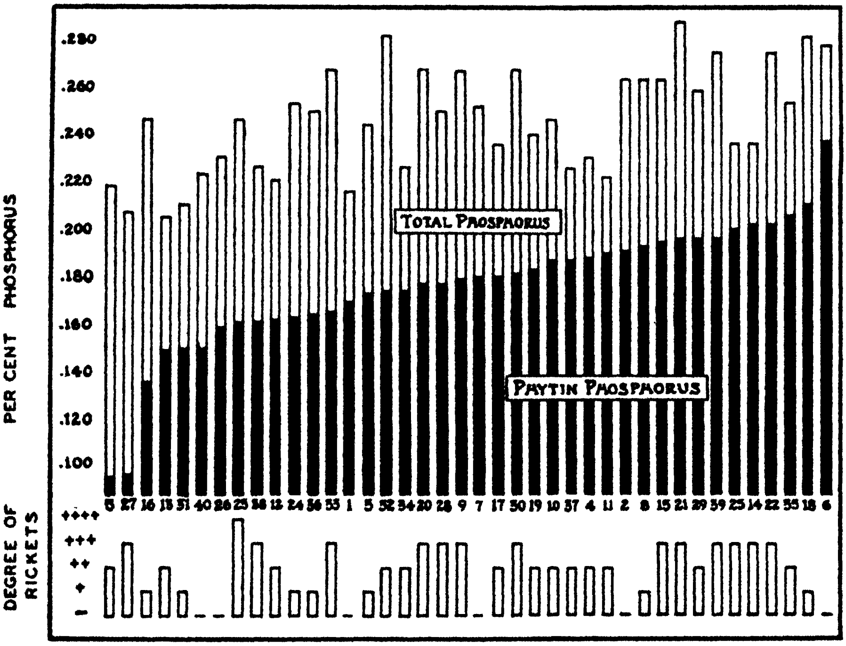

Fig. 1 Relation of corn phytin phosphorus in the diet as fed to the degree of rickets produced.

its low assimilability. For the sake of accuracy, it should be pointed out that inositol hexaphosphoric acid occurs in grains in the form of phytin, a salt of the hexaphosphoric ester of inositol (Posternak, '19) and that associated with this substance there occurs naturally the enzyme phytase which under suitable moisture and temperature conditions catalyzes the hydrolysis of phytin, yielding inorganic salts of orthophosphoric acid. In sprouting grains phytin is split to inorganic phosphates and inosite, presumably through the activity of phytase (DeTurck et al., '33).

Bruce and Callow reduced the anticalcifying properties of oatmeal and other cereals by a treatment with hydrochloric acid and assumed the improvement to be due to hydrolysis of inositol hexaphosphoric acid. They state, in regard to the unexplained variations in severity of rickets induced from time to time by a rachitogenic diet, in spite of its reasonably careful control, that "one possible factor in this variation may be the proportions of phytin and other phosphorus in samples of maize of similar phosphorus content," a view which we entertained at the beginning of our work. Analysis of our data fails to demonstrate that either absolute or proportional amount of phytin phosphorus in the Steenbock no. 2965 diet correlates with the degree of rickets obtained.

The findings of Bruce and Callow appear to substantiate the generally accepted view expressed by Plimmer ('13 a, '13 b) that phosphorus in the form of phytin is poorly available in the nutrition of carnivores and herbivores. An important part of the evidence of these authors is based on the finding that acid treated oatmeal shows an apparent greater availability of phosphorus (at least, less anticalcifying tendency) than the untreated cereal, and the assumption is natural that this improvement is related to the acid hydrolysis of phytin with a resultant increase in the proportion of inorganic phosphates which, under suitable conditions, have been shown by many workers to be available for calcification processes.

The finding of Templin and Steenbock ('33 a, '33 b) that germinated corn is rendered definitely less rachitogenic by autolysis, during which process it may be assumed that phytin phosphorus is actively hydrolyzed, is concordant with the above view of Plimmer. A previous report by these authors that unripe maize is less rachitogenic than the fully ripened grain is also suggestive, since DeTurck ('33) has demonstrated that phytin is formed at or near the point of storage (of phosphorus) in the ripening of seeds. He also quotes Zaleski in Kostychev's 'Plant physiology' ('31) as pointing out that during the ripening of seeds, a considerable amount of phosphorus enters the seed in the form of . . . . phytin. Quantitative data on the relative proportions of phytin and non-phytin phosphorus in germinated and hydrolyzed grains seem to be necessary in order that their real bearing upon the general question of availability of phosphorus may be established.

It would appear at first glance that the work reported by us previously on the increase of rachitogenic properties of corn stored after grinding in loosely covered containers for a period of months is not in accord with the theory that phytin phosphorus is less available than the inorganic derivatives of its hydrolysis. It would seem that storage might permit phytase action through which the availability of phosphorus would be increased and rachitogenic property decreased. The relatively dry state of our corns during storage permitted relatively feeble phytase action, although not prohibiting oxidation of classical vitamin D. One may presume that any decrease in the rachitogenicity of stored corn, attributable to a reduction of phytin, is outweighed by other changes, the net result of which is the observed increase in rachitogenic effect. The apparent discrepancy between our findings and the theory of relative non-availability of phytin then disappears.

While it is important to consider the matter of phytin phosphorus in materials used for compounding a rachitogenic diet, our results strengthen our opinion that too much emphasis must not be laid upon this factor alone; that there are other factors contributing to the effects of such diets which may completely overshadow the alleged phytin effect. Perhaps undue attention is being paid to the matter of phosphorus in such diets, but it is necessary to evaluate this factor as well as others before the real dietary criteria of rachitogenicity can be established. A continuation of an investigation of other groups of phosphorus in corn is proposed in order that any demonstrable differences in their availability in the rachitogenic diet may be determined.

CONCLUSIONS

The variations in rachitogenic properties of forty corn samples used in the Steenbock no. 2965 ration do not correlate with the absolute or relative amounts of phytin phosphorus, nor with the 'non-phytin phosphorus' fraction of these corns, nor with the total Ca: total P ratio of rations as fed.

It has been shown that the content of inositol hexaphosphoric acid (salts) is not the sole cause of the anticalcifying action of all cereals. In the case of corn, at least, there exists some other important factor or factors.

Until the unidentified rachitogenic factors of corn are understood and controllable, the incautious use of this ingredient in a 'standardized' rachitogenic diet may be expected to produce discordant results in the bio-assay of vitamin D.

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PRODUCTION AND COMPOSITION OF SOW'S MILK

E. H. HUGHES AND HUGH G. HART
Division of Animal Husbandry, College of Agriculture, University of California, Davis

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Information concerning milk production of the sow, the composition of sow's milk, and the constituents of its ash is limited. A more complete knowledge of sow's milk was needed, to give a fuller understanding of the nutritional requirements of the fast-growing pig and to aid in formulating suitable rations. The available information has been collected and to it has been added data obtained at this station.

MILK PRODUCTION

Production records of sixty sows and seventy-six lactations are presented in table 1. The average daily and, where possible, total production for one lactation are shown. The sows from which the records were obtained varied in age and weighed from 250 to 500 pounds each.

These data are reported for the years 1865 and 1933, inclusive. As would be expected, there are wide variations in the average daily and total milk production. The lowest production was obtained by von Gohren (1865) and the highest by Ostertag and Zuntz ('08). The daily production reported by von Gohren (1865) is far below the average and is given for its historical interest. The average daily production for seventy-five lactations was 6.8 pounds. Henry and Woll (1897) reported the milk yield was not large immediately after farrowing and at weaning time—that the maximum flow was in the middle of lactation; Davies ('04) found a gradual increase in production to the sixth week after parturition and a gradual decrease thereafter; and Carlyle ('03) states that some sows yield nearly double that of others. Schmidt and Lauprecht ('26) report that sows fed a high-protein diet produce more milk than those on a low-protein ration; that sows suckling 8.5 or more pigs yield more than those having fewer pigs, and that sows suckling their third to sixth litters produce more milk than sows in their first and second lactations. They found, too, that sows with a higher protein diet gave much more milk in the middle of lactation than those getting less protein; that older sows produce more milk in the early part of lactation, whereas the young sows gave the same amount in the late as in the first part of the period.

TABLE 1

Milk production of sows

NUMBER OF SOWS	AVERAGE NUMBER OF PIGS IN LITTER	INDIVIDUAL DAILY PRODUCTION	PRODUCTION DURING LACTATION			REPORTED BY
			8 weeks duration	10 weeks duration	12 weeks duration	
		pounds	*pounds*	*pounds*	*pounds*	
1	...	3.4	....	....	....	von Gohren (1865)
4	6.8	5.2	....	364.0[2]	....	Henry and Woll (1897)
12	7.2	5.4	....	....	464.9	Carlyle ('03)
1	7.0	5.6	....	389.2	....	Davies ('04)
3	...	11.4	638.4	....	....	Ostertag and Zuntz ('08)
1	9.0	10.2	....	....	....	Schmidt and Lauprecht ('26)
1	7.0	11.6	....	....	....	
1	9.0	7.2	404.9	....	....	
1	8.0	8.2	460.5	....	....	
1	6.0	10.7	600.0	....	....	
20[1]	8.3	6.9	388.2	....	....	
4	...	6.9	....	481.0	....	Ohligmacher[3]
7	...	6.5	....	457.7	....	Ohligmacher[3]
2	7.5	7.7	....	539.0	....	The authors
Average	*7.9*	*6.8*	*413.2*	*447.3*	*464.9*	

[1] Thirty-six lactations.

[2] Calculated from daily production.

[3] Reported by Schmidt and Lauprecht ('26).

The authors found, for the two sows tested, their maximum production was attained between the second and fourth weeks of lactation and that their total production was nearly the same. In 10 weeks one sow produced 553 pounds and the other 525 pounds of milk. These figures are above the average of other production records reported.

METHODS OF OBTAINING SAMPLES FOR ANALYSIS AND TOTAL YIELD

Some difficulty was experienced in obtaining milk samples. Little success was realized until the milk-secreting habit of the sow was understood. It was found that she does not give her milk 'down' continuously while the pigs are suckling, but there seemed to be a definite succession of events in the process. The sow may allow the pigs to suckle for some time without giving milk. After sufficient stimulation by the rooting, squealing pigs she begins grunting in a characteristic manner, easily understood and recognized by an experienced operator. Suddenly the milk starts coming and flows freely. The pigs cease their rooting and clamoring, settle back and suckle. This continues for about 60 seconds when the milk flow ceases as suddenly as it began.

Considering these facts, the operator must govern himself accordingly. He proceeds to the sow quietly so that she is not disturbed. When the milk flow begins he must work quickly, keeping a pig away from the teat from which the sample is being taken. Samples of 30 to 50 cc. are often obtained from a single gland.

Samples of colostrum are more readily obtained than normal milk. During parturition and before the newborn pigs suckle, the operator proceeds quietly to the sow and obtains a sample in a similar manner as one would milk a cow. Pressure exerted approximately in the center of the mammary gland system seems to aid in making the colostrum flow more freely. After the samples were drawn they were immediately placed in an ice box and kept for analysis. Attempts were made to obtain samples by using a breast pump and a small milking machine with poor success.

The total production of a sow was obtained by allowing the pigs to suckle at definite intervals of time for a 24-hour period. The pigs were weighed before and after suckling and the increase in weight was taken as a measure of the milk secreted for that period. This was done every 10 days and was considered the average yield for the 10 days. In the early stages of lactation the pigs were allowed to suckle every 2 hours; later during the production test each 4 hours; and near the end of lactation the intervals were increased to 6 hours each.

METHODS OF ANALYSIS

Total solids were obtained by evaporation on a steam bath and then drying in an oven at about 90°C.; ash was determined by ashing the dried sample in an electric muffle at about 500°C. More difficulty was experienced in ashing colostrum than normal milk. Calcium was determined by dissolving the ash in dilute HCl and transferring to a volumetric flask. Various sizes of volumetric flasks were used, according to the weight of the ash. Roughly, the desired concentration was about 0.04 gm. ash in a 50 cc. volumetric flask. An aliquot of 2 cc. was taken and put into a 15 cc. centrifuge tube. To be sure the solution was at the correct pH, brom cresol purple was used as an indicator. From this point the Clark-Collip ('25) modification of the method of Kramer and Tisdall for determining the serum calcium of blood was used. Phosphorus was obtained by using a 1 cc. aliquot of the same solution, after which the procedure used was the same as in the method of Fiske and Subbarow ('25) for determining the inorganic phosphorus in blood serum after the blood proteins had been precipitated. Fat was determined by the Babcock test, except when the sample was small, in which case the ether extract method was employed. Protein was found by obtaining the nitrogen by the Kjeldahl method and multiplying by the factor 6.38.

COMPOSITION OF SOW'S MILK

The composition of colostrum and normal milk was studied. Colostrum was considered to be that produced during the first 60 hours after parturition and normal milk that produced from 96 hours after farrowing until the end of lactation. Between these designated times, a change takes place in the composition of the secretion that has been termed 'transition milk.' The percentage composition of these types of milk as presented by other workers is presented in tables 2 and 3. The percentage of total solids and protein of colostrum in table 2 are much higher than in normal sow's milk in table 3; and while the fat content varied within wide limits, the average was 6.35 per cent., Ostertag and Zuntz ('08) found the albumin and globulin content of colostrum was exceedingly high, being nearly twice that of the casein. They reported casein as 4.69 per cent and the albumin and globulin taken together as 9.09 per cent. Hempel and Ohligmacher[1] obtained a fat content of 7.38 per cent from ten sows with their first and second litters, while nine sows in their third and sixth lactations showed a content of 5.38 per cent. Hempel reported the fat content of colostrum taken from any one nipple varied little in an individual sow. Ohligmacher found the protein

TABLE 2

Composition of colostrum

NUMBER OF SOWS	TOTAL SOLIDS	FAT	PROTEIN[1]	MILK SUGAR	ASH	REPORTED BY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	29.87	9.53	15.56	3.84	0.85	von Gohren (1865)
2	24.66	4.04	15.86	1.77	0.71	Ostertag and Zuntz ('08)
19	....	6.43	....	....	...	Hempel and Ohligmacher[2]
11	....	...	16.27	...	...	Ohligmacher[2]
Average	*26.40*	*6.35*	*16.16*	*2.46*	*0.76*	

[1] In arriving at the amount of protein von Gohren and Hempel and Ohligmacher used the factor 6.25, while Ostertag and Zuntz used 6.37.

[2] Reported by Schmidt and Lauprecht ('26).

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[1] Reported by Schmidt and Lauprecht ('26).

content of colostrum was 16.27 per cent; that the percentage of protein falls rapidly—at the end of 2 days after parturition the average of three sows was 7.25 per cent. The same worker found four sows during a 10 weeks' lactation period fed a high-protein diet produced milk that contained 7.83 per cent fat and 7.56 per cent protein, whereas seven sows fed a low protein diet gave milk with 6.63 per cent fat and 5.15 per cent protein. Normal sow's milk was found to contain (by these workers) 18.18 per cent total solids, 6.85 per cent fat, 6.19 per cent protein, 5.00 per cent milk sugar and 0.98 per cent fat. The average specific gravity of thirty-seven samples found reported was 1.0402.

TABLE 3

Composition of normal milk

NUMBER OF SOWS	NUMBER OF SAMPLES	TOTAL SOLIDS	FAT	PROTEIN[1]	MILK SUGAR	ASH	REPORTED BY
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
2	2	13.87	1.48	7.91	2.65	1.14	Scheven[3]
1	2	15.15	2.98	9.29	2.19	0.79	von Gohren (1865)
1	1	17.07	6.88	6.89	2.01	1.29	Lintner[3]
2	3	18.23	5.59	5.89	5.58	0.87	Cameron[3]
1	1	17.54	9.23	5.09	1.68	1.53	Ivon[3]
12	17	....	7.56	...	...	...	Petersen and Oetken (1896 a)
8	9	....	5.03	...	...	...	Petersen and Oetken (1896 b)
..	22	....	6.59	...	...	...	Petersen and Oetken (1897)
4	7	19.08	7.06	6.20	4.75	1.07	Henry and Woll (1897)
5	5	17.72	5.97	5.12	5.81	0.82	Woll (1899)
1	1	14.58	4.55	5.60	3.34	1.09	Ramont and Braun[3]
12	24	19.49	6.89	6.06	5.64	0.98	Carlyle ('03)
1	1	16.74	5.41	4.76	5.49	1.08	Davies ('04)
2	6[2]	15.84	5.01	5.54	4.59	0.63	Ostertag and Zuntz ('08)
1	1	....	3.68	7.93	4.00	...	Folin, Dennis and Minot ('19)
..	354	....	6.99	...	...	...	Hempel and Ohligmacher[4]
..	66	....	...	6.25	...	...	Ohligmacher[4]
Aver.		*18.18*	*6.85*	*6.19*	*5.00*	*0.98*	

[1] In arriving at the amount of protein, Hempel and Ohligmacher and Ohligmacher used the factor 6.25, Ostertag and Zuntz 6.37.

[2] Analyses by Ostertag and Zuntz based on six samples for total solids and fat; five for protein; and one for milk sugar and ash.

[3] Reported by König ('03).

[4] Reported by Schmidt and Lauprecht ('26).

In table 4 there is presented the analysis of colostrum and normal sow's milk as obtained in 1933 and 1934 at this station. The figures of the various components of colostrum vary somewhat from those in table 2; however, these differences, except for the percentage of fat, might be explained on the variation in time of taking the samples. Of the total number of samples of fat reported in table 2, nineteen were by Ohligmacher. The sows he was working with no doubt were higher fat producers than those in this station's herd.

TABLE 4

Percentage composition of colostrum and normal sow's milk as determined at the California Experiment Station

TIME OF TAKING SAMPLE	NUMBER OF SOWS	TOTAL SOLIDS	FAT	PROTEIN[2]	ASH
		Colostrum			
During parturition	13	31.86 (13)[1]	5.14 (7)	16.64 (7)	0.61 (13)
10 to 40 hours after parturition	7	23.50 (10)	5.47 (6)	12.58 (6)	0.69 (10)
Average		*28.23*	*5.29*	*14.77*	*0.64*
		Normal milk			
4 to 7 days after parturition	13	18.39 (18)	5.50 (9)	5.57 (9)	0.77 (18)
15 to 45 days after parturition	8	16.58 (20)	4.90 (10)	5.95 (10)	0.99 (20)
50 to 80 days after parturition	7	18.76 (12)	5.61 (6)	8.13 (6)	1.20 (12)
Average		*17.76*	*5.29*	*6.34*	*0.96*

[1] The numbers in parenthesis indicate the number of samples available to arrive at the average figure.

[2] The factor 6.38 was used in determining the percentage of protein.

Attention is called to the rapid change in the colostrum milk, particularly in the percentage of total solids and protein. Samples drawn during parturition contained 31.86 per cent total solids and 16.64 per cent of protein, while an average of milk taken from 10 to 40 hours later contained 23.50 per cent total solids and 12.58 per cent protein. There was a slight increase in the per cent of ash after parturition. Colostrum was found to be higher in total solids and protein, lower in ash, and slightly lower in fat than normal milk.

The percentage composition of normal milk as obtained at this station, given in table 4, is similar to the average of those reported by other workers in table 3, except that the fat content is materially lower. The individual differences of the sows under test probably account for this.

TABLE 5

Composition of the ash of colostrum and normal milk of the sow as determined at the California Experiment Station

TIME OF TAKING SAMPLE	NUMBER OF SOWS	NUMBER OF SAMPLES	ASH IN PER CENT	ASH AS PER CENT OF TOTAL SOLIDS	CALCIUM AS PER CENT OF ASH	PHOSPHORUS AS PER CENT OF ASH	CALCIUM AS PER CENT OF MILK	PHOSPHORUS AS PER CENT OF MILK	CALCIUM-PHOSPHORUS RATIO
Colostrum									
During parturition	13	13	0.61	1.97	9.17	12.99	0.055	0.078	1: 1.42
10 to 40 hours after parturition	7	10	0.69	2.93	15.33	13.14	0.104	0.089	1: 0.86
Average			*0.64*	*2.39*	*11.85*	*13.06*	*0.076*	*0.083*	*1: 1.18*
Normal milk									
4 to 7 days after parturition	13	18	0.77	4.22	24.79	15.81	0.194	0.122	1: 0.64
15 to 45 days after parturition	8	20	0.99	6.02	25.96	15.36	0.257	0.152	1: 0.59
50 to 80 days after parturition	7	12	1.20	6.37	27.67	16.22	0.331	0.194	1: 0.59
Average			*0.96*	*5.46*	*25.95*	*15.63*	*0.252*	*0.151*	*1: 0.61*

COMPOSITION OF THE ASH

The total ash and the calcium and phosphorus content of twenty-three samples of colostrum and of fifty samples of normal sow's milk were determined. The average figures for these determinations as made at this station are reported in table 5. An examination of this table reveals certain definite differences in the total ash as well as differences in total amounts of calcium and phosphorus between colostrum and normal milk. There was a significant change in the calcium and phosphorus ratio and in the relation of ash to total solids between the milk given early and that produced later in the lactation period.

The total ash and the ash figured as per cent of total solids, increase steadily during lactation. The content of calcium in colostrum taken during parturition is only about one-third that of normal milk. It increases rapidly during the first few days of lactation. While there is less phosphorus in colostrum than in normal milk, the difference is only a slight one as compared to the difference in calcium concentration. Milk taken during parturition actually contained more phosphorus than calcium. The average of eight samples, taken 10 to 40 hours later, shows a rapid increase in calcium; however, two of the eight samples taken about 20 hours after parturition still showed a higher phosphorus than calcium content. The calcium and phosphorus content of the milk and similarly of the ash showed a slight increase late in lactation. The calcium-phosphorus ratio as found in these studies was 1 : 1.18 for colostrum and 1 : 0.61 for normal milk.

DISCUSSION

A comparison of the average composition of the milk of the sow with those reported by the associates of Rogers ('28) for the milk of various mammals shows sow's milk to be higher in total solids than that of the human, cow, and horse, similar to that of the goat, and lower than that of sheep. In protein content it exceeds that of any of the species reported; the fat content is comparable to that of the sheep and the goat, but is in excess of the milk of the human, cow, and horse. The lactose or milk sugar content is similar in these species. The ash content is about the same as that of the sheep, slightly greater than that of the goat, about one-third greater than that of the cow, and about three times greater than that of human and mare's milk. In composition the normal milk of the sow is more comparable to that of the ewe than of the other mammals. The young of both species double their birth weight in a relatively short time which may in part be a reason for this similarity. Changes occurring from colostrum to normal milk are similar in the human, the horse (Linton, '31), the cow and the sow. Soon after parturition there is a distinct and decided decrease in the total solids and protein of the species mentioned. In the cow, mare and sow the change is rapid. As lactation progresses there seems to be a slight increase in percentage of fat of the milk of the mare and sow, with a decrease in cow's milk. In the material reported, with the exception of the milk of the sow, there is a slight and steady decrease in the ash content. The ash of sow's milk is low in colostrum and increases slightly from colostrum to normal milk and continues to increase at a slow rate throughout the lactation period.

Richmond ('20) reports that the ash of cow's milk contains 14.48 per cent calcium and 12.81 per cent phosphorus, that calcium in the milk varies from 0.094 to 0.150 per cent and phosphorus from 0.084 to 0.128 per cent. Babcock and Leach ('20) have shown the calcium and phosphorus content of the ash of the milk of the cow to be, respectively, 14.30 per cent and 10.61 per cent. Widdows et al. ('30), working with human milk, reported that an average of 154 samples contained 0.035 gm. of calcium in 100 cc. of milk and seventy-three samples contained 0.018 gm. of phosphorus in the same quantity. They show an increase in the calcium and phosphorus from the first week to the second and fourth months and then a decrease, particularly in the calcium. They found an ash content in 100 cc. of milk of 0.289 gm. during the first 2 weeks, 0.243 gm. from the second to the fourth month and 0.181 gm. from the eighth to the twelfth month, a gradual decrease during the early and a rapid decrease in the latter part of lactation.

While the total solids and protein of sow's milk fall rapidly immediately after parturition, the percentage of ash begins to increase and continues to do so through lactation. During parturition the calcium content of the ash of colostrum, when it is compared with that of normal milk, is exceedingly low and it immediately increases in percentage with the decrease of total solids and protein. The percentage of phosphorus in the ash is lower in colostrum than in normal milk, but the difference is slight compared to that of calcium. The ratio of calcium to phosphorus as determined in these studies changes rapidly from a phosphorus content in excess of that of calcium during parturition to a ratio of about 1:0.60 about a week later, the ratio remaining constant thereafter.

SUMMARY

Sows produce about 6.8 pounds of milk per day for an average lactation period of 8 to 12 weeks.

The averages for the constituents of colostrum as determined in these studies were: total solids 28.02 per cent, fat 5.96 per cent, protein 15.49 per cent, and ash 0.65 per cent.

The averages for normal milk as obtained were: total solids 17.98 per cent, fat 6.77 per cent, protein 6.22 per cent, and ash 0.97 per cent.

The average calcium and phosphorus content of colostrum of sow's milk as found at this station were 0.076 per cent and 0.083 per cent, respectively; while for normal milk they were: calcium 0.252 per cent and phosphorus 0.151 per cent.

Like cow's milk, the milk of the sow, at parturition, contains large quantities of total solids and protein, but both fall rapidly immediately thereafter.

The ash content of sow's milk is relatively low at parturition and increases during lactation.

The calcium and phosphorus content of normal sow's milk is higher than that reported for the human or the cow.

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MINERAL EXCHANGES OF MAN

V. BALANCES OF ELECTROLYTES IN A CASE OF HYPERPARATHYROIDISM

SAMUEL H. BASSETT[1]

Department of Medicine, School of Medicine and Dentistry, University of Rochester and Medical Clinic of Strong Memorial Hospital, Rochester, New York

FIVE FIGURES

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The disordered metabolism of calcium and phosphorus in hyperparathyroidism has been studied intensively since Mandl ('26) found that a case of generalized osteitis fibrosa (Recklinghausen) was improved by removal of a parathyroid tumor. The literature dealing with metabolic and other phases of the subject has been reviewed by Barr and Bulger ('30) Hunter ('31) and by Jaffe ('33).

Criteria upon which a presumptive diagnosis of parathyroid tumor may be made are now well recognized. Briefly stated, these are: hypercalcemia, hypophosphatemia, hypercalcinuria and x-ray evidence of generalized bone disease which in the milder or earlier cases may be nothing more than thinning of the bones and osteoporosis, while in the more advanced stages multiple bone cysts or giant cell tumors occur.

The opportunity to study some aspects of the metabolism of inorganic salts in a patient with osteitis fibrosa, from whom a parathyroid tumor was subsequently removed at operation prompted the present report. The details of the clinical history have been given in a separate communication (Morton, '33) and need not be repeated here, except to state that the diagnostic criteria mentioned in the preceding paragraph were fulfilled.

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METHODS

Balance studies were conducted in the metabolism unit of the hospital under carefully controlled conditions. During the greater part of the experiment the diet received by the patient was sampled and analyzed in order to determine intake. The methods employed in analysis of food and excreta have been reported in detail previously (Bassett, Elden and McCann, '31; Bassett and Van Alstine, '35). Calcium of the serum was determined by Clark and Collip's ('25) modification of the Kramer and Tisdall procedure; inorganic phosphorus was determined by the method of Kuttner and Cohn ('27). Metabolic periods were 6 days in length. Balances of nitrogen, calcium, magnesium, sodium, potassium, phosphorus and chlorine were computed for each period.

Two types of diet were used. The first contained an abundant supply of calcium and phosphorus; the second was deficient in calcium, but contained a moderate amount of phosphorus. By causing the patient to ingest each of these diets in turn for a sufficient length of time, it was hoped that the severity of the metabolic disturbance could be evaluated and that the information thus obtained would serve as a guide for subsequent therapy. Average daily intakes of protein, carbohydrate, fat, calcium and phosphorus; the Ca:P ratio; excess of acid or base in the ash of the food and calories for each diet are recorded in table 1. In order to avoid any marked gain or loss in weight, the caloric intake was adjusted to the energy expenditure of the patient in such a manner that it provided for her basal requirement and allowed a sufficient surplus to permit a moderate amount of bodily activity. Weight fluctuated within a comparatively narrow range (48.5 to 51.6 kg.). Her general health was excellent at all times.

METABOLISM OF CALCIUM AND PHOSPHORUS

Balances of calcium and phosphorus during periods in which the high and low calcium diets were ingested are shown in figure 1. Each element has been expressed in equivalents of

N/10 solution, in order that the rather close relationship between retention of calcium and phosphorus in the body or their loss from it may be more clearly appreciated. In constructing figure 1, the phosphorus balance has in each instance been corrected for retention or loss of phosphorus with nitrogen after the method described by Aub et al. ('29). The partition of calcium and phosphorus between urine and feces is shown in the data assembled in table 2. During control periods the retentions of both elements diminished in successive periods, although the intake of each remained essentially constant.

TABLE 1

Average daily intake of food, calcium and phosphorus, including Ca:P ratios and the estimated excess of acid or base in the ash of the food

DIET	PROTEIN	CARBO-HYDRATE	FAT	CALORIES	CALCIUM	PHOSPHORUS	RATIO Ca : P	EXCESS ACID[1] OR BASE ML. N/10	REMARKS
	gm.	*gm.*	*gm.*		*gm.*	*gm.*			
High calcium	78	178	109	2059	1.42	1.80	0.79	Base + 214	Control periods 11, 12, 13, 17 and 18
	78	178	109	2059	1.42	3.87	0.36	Acid + 319	Sodium acid phosphate periods 14, 15 and 16
Low calcium	50	179	137	2226	0.148	0.706	0.21	Acid + 164	Controls periods 19, 20 21, 25 and 26
	50	179	137	2226	1.48	2.80	0.53	Acid + 38	Calcium salts and acid phosphate—periods 22, 23 and 24

[1] The acidifying effect of ingestion of sodium acid phosphate and the alkalinizing effect of calcium lactate and gluconate have been included in estimating the excess of acid or base in the food. The high Ca and P intake shown in periods 22, 23 and 24 was due to superposition of calcium lactate, calcium gluconate and sodium acid phosphate on the low calcium diet.

Effect of ingestion of $NaH_2PO_4.H_2O$ *on balance.* The addition of sodium acid phosphate to the diet in the amounts indicated (fig. 1 and table 2) caused a considerable increase in the retention of both elements. The immediate effect was to produce a large increase in the retention of phosphorus in excess of the amount which could have combined with

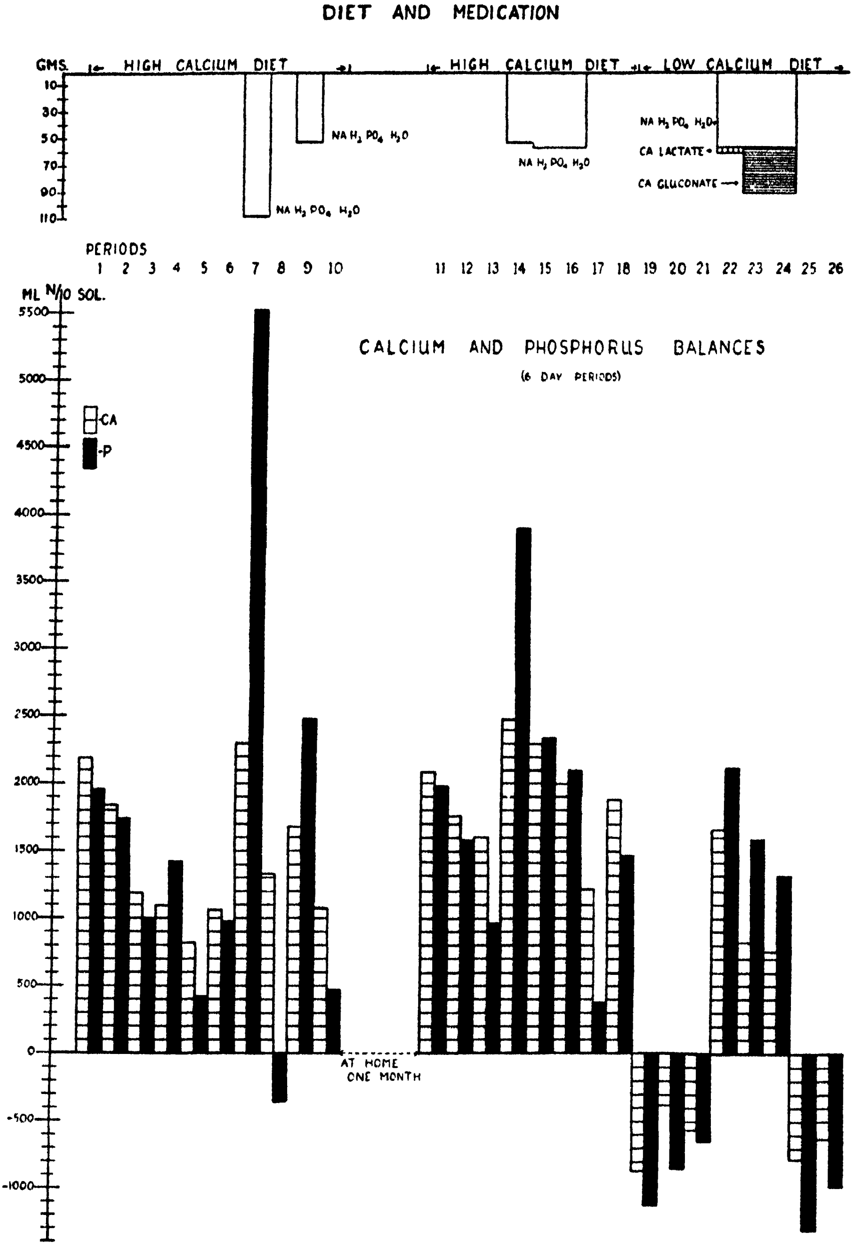

Fig. 1 Only the amounts of calcium or phosphorus retained in or lost from the body are shown in the graph. Phosphorus has been considered trivalent and each balance has been corrected for phosphorus presumably retained or lost with nitrogen (Aub et al., '29).

calcium to form $Ca_3(PO_4)_2$ and to decrease the excretion of calcium in the urine. When the administration of acid phosphate was continued in consecutive periods (14 to 16) the excretion of phosphorus in the urine gradually increased and the retentions of phosphate decreased. In the first control period which followed (period 17) comparatively little phosphorus was retained and the retention of calcium also decreased, but not to a similar extent. The inequalities in the retention of calcium and phosphorus in individual periods show that either one may be retained for a time in excess of the other. However, when the total retentions of each element were compared over a longer interval of time (periods 11 to 18, inclusive) it was found that 30.78 gm. of calcium and 15.27 gm. of phosphorus (excluding phosphorus deposited with nitrogen) were retained. The Ca:P ratio was $\frac{30.79}{15.27} =$ 2.01 which is almost identical with the Ca:P ratios found in bone.

Effect of sodium acid phosphate on concentrations of calcium and inorganic phosphorus in the serum. Hypercalcemia was at no time extreme; on the other hand, very low values of inorganic phosphorus in serum were frequently observed (table 3). As Bulger, Dixon, Barr and Schregardus ('30) and Albright, Bauer, Claflin and Cockrill ('32) have shown, the high values of calcium could be reduced toward normal and the low values of inorganic phosphorus were increased by the administration of acid phosphate. These effects, as Albright and collaborators noted, were dependent upon the amount of acid phosphate administered. Large doses of phosphate (period 7) caused the inorganic phosphorus of the serum to rise and the level of calcium in serum to fall nearly to normal. When approximately half of this large dose was employed (period 9) the effect on the levels of Ca and P in the serum was less pronounced. In both instances there was increased retention of calcium and phosphorus in the body; the larger dose produced the more marked retention. In general, then, the effects of administration of acid phosphate were similar to those noted by others who have

TABLE 2

Exchanges of nitrogen, calcium, phosphorus and magnesium

PERIODS (6 DAYS)	NITROGEN			CALCIUM			PHOSPHORUS			MAGNESIUM			COMMENT
	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
11	75.33	U. 64.35 F. 5.05 T. 69.40	+ 5.93	8.50	U. 3.33 F. 0.98 T. 4.31	+ 4.19	10.50	U. 6.90 F. 1.25 T. 8.15	+ 2.35	1.723	U. 0.889 F. 0.635 T. 1.524	+ 0.199	Control period High calcium diet
12	75.41	U. 68.47 F. 3.52 T. 71.99	+ 3.42	8.43	U. 3.64 F. 1.27 T. 4.91	+ 3.52	10.30	U. 7.21 F. 1.28 T. 8.49	+ 1.81	1.668	U. 0.863 F. 0.674 T. 1.537	+ 0.131	Control period High calcium diet
13	75.09	U. 66.90 F. 4.63 T. 71.53	+ 3.56	8.19	U. 3.41 F. 1.57 T. 4.98	+ 3.21	10.05	U. 7.16 F. 1.70 T. 8.86	+ 1.19	1.668	U. 0.828 F. 0.691 T. 1.519	+ 0.149	Control period High calcium diet
14	76.94	U. 68.87 F. 3.90 T. 72.77	+ 4.17	8.11	U. 2.17 F. 0.98 T. 3.15	+ 4.96	22.87	U. 17.24 F. 1.39 T. 18.63	+ 4.24	1.676	U. 0.691 F. 0.648 T. 1.339	+ 0.337	As above + 52 gm. $NaH_2PO_4.H_2O$
15	77.06	U. 69.35 F. 4.92 T. 74.27	+ 2.79	8.36	U. 2.15 F. 1.60 T. 3.75	+4.61	23.43	U. 18.72 F. 2.15 T. 20.87	+ 2.56	1.668	U. 0.712 F. 0.943 T. 1.655	+ 0.013	As above + 56 gm. $NaH_2PO_4.H_2O$
16	79.25	U. 68.95 F. 3.45 T. 72.40	+ 6.85	8.33	U. 2.42 F. 1.83 T. 4.25	+ 4.08	23.44	U. 18.90 F. 2.03 T. 20.93	+ 2.51	1.701	U. 0.706 F. 0.844 T. 1.550	+ 0.151	As above + 56 gm. $NaH_2PO_4.H_2O$
17	79.44	U. 66.80 F. 3.86 T. 70.66	+ 8.78	8.60	U. 4.42 F. 1.75 T. 6.17	+ 2.43	11.16	U. 8.56 F. 1.75 T. 10.31	+ 0.85	1.643	U. 0.911 F. 0.790 T. 1.701	— 0.058	Control period High calcium diet

18	78.75	U. 69.09 F. 3.07 T. 72.16	+ 6.59	9.63	U. 4.50 F. 1.34 T. 5.84	+ 3.79	10.99	U. 7.91 F. 1.22 T. 9.13	+ 1.86	1.748	U. 0.872 F. 0.541 T. 1.413	+ 0.335	Control period High calcium diet
19	55.39	U. 61.41 F. 2.26 T. 63.67	— 8.28	0.75	U.1.62 F. 0.85 T. 2.47	— 1.72	4.32	U. 4.88 F. 1.00 T. 5.88	— 1.56	1.124	U. 0.546 F. 0.640 T. 1.186	— 0.062	Control period Low calcium diet
20	57.81	U. 51.63 F. 3.87 T. 55.50	+ 2.31	1.02	U. 1.65 F. 0.12 T. 1.77	— 0.75	4.42	U. 4.43 F. 0.75 T. 5.18	— 0.76	1.204	U. 0.476 F. 0.703 T. 1.179	+ 0.025	Control period Low calcium diet
21	56.48	U. 50.66 F. 4.13 T. 54.79	+ 1.69	0.90	U. 1.80 F. 0.21 T. 2.01	— 1.11	4.25	U. 4.13 F. 0.70 T. 4.83	— 0.58	1.150	U. 0.479 F. 0.676 T. 1.155	— 0.005	Control period Low calcium diet
22	56.13	U. 47.04 F. 3.46 T. 50.50	+ 5.63	9.18	U. 1.35 F. 4.50 T. 5.85	+ 3.33	16.68	U. 12.07 F. 2.14 T. 14.21	+ 2.47	1.152	U. 0.341 F. 0.696 T. 1.037	+ 0.115	As above + 60 gm. calcium lactate + 56 gm. $NaH_2PO_4.H_2O$
23	54.73	U. 52.65 F. 3.16 T. 55.81	— 1.08	8.77	U. 1.30 F. 5.81 T. 7.11	+ 1.66	16.90	U. 13.25 F. 2.05 T. 15.30	+ 1.60	1.208	U. 0.403 F. 0.720 T. 1.123	+ 0.085	As above + 90 gm. calcium gluconate + 56 gm. $NaH_2PO_4.H_2O$
24	54.49	U. 52.90 F. 3.28 T. 56.18	— 1.69	8.74	U. 1.22 F. 6.00 T. 7.22	+ 1.52	16.82	U. 13.26 F. 2.26 T. 15.52	+ 1.30	1.172	U. 0.384 F. 0.641 T. 1.025	+ 0.147	As above + 90 gm. calcium gluconate + 56 gm. $NaH_2PO_4.H_2O$
25	54.20	U. 52.36 F. 2.95 T. 55.31	— 1.11	0.92	U. 1.64 F. 0.84 T. 2.48	— 1.56	4.20	U. 4.87 F. 0.73 T. 5.60	— 1.40	1.188	U. 0.526 F. 0.577 T. 1.103	+ 0.085	Control period Low calcium diet
26	55.29	U. 49.04 F. 3.06 T. 52.10	+ 3.19	0.79	U. 1.70 F. 0.31 T. 2.01	— 1.22	4.14	U. 4.17 F. 0.81 T. 4.98	— 0.84	1.172	U. 0.536 F. 0.739 T. 1.275	— 0.103	Control period Low calcium diet

Legend: U = urine, F = feces, T = total. Signs: + retention in body; — loss from body.

used salts of orthophosphoric acid in this condition. The only unusual feature of this phase of the experiment appears to have been the large amounts of calcium and phosphorus which were retained when these elements were furnished in rather high concentrations in the diet. Inspection of table 2 shows that from one-third to one-half of the calcium ingested during periods 11 to 18 was deposited in the body.

Effect of a low calcium diet. The effect of decreasing the calcium of the food from 1.42 gm. to 0.15 gm. per diem resulted as had been expected in a loss of calcium, presumably from the bones. The phosphorus balance ran essentially parallel with the calcium balance, although there was no absolute deficit of phosphorus in the food (table 1). A more surprising feature of this phase of the experiment was the fact that more phosphorus was lost than the loss of calcium seemed to warrant, assuming, of course, that these elements were being withdrawn from the bones. This excess loss of phosphorus was not due to a negative nitrogen balance, since

TABLE 3

PERIOD 6 DAYS	Ca IN SERUM MG./PER CENT	INORGANIC P IN SERUM MG./PER CENT	TOTAL SERUM PROTEIN GM./PER CENT	Ca × P PRODUCT	CALCIUM BALANCE, GM.	COMMENT
2	13.30 (6)	2.38 (6)	7.66 (6)	31.6 (6)	+ 3.66	High calcium-phosphorus diet
4	13.66 (6)	1.57 (6)	6.51 (6)	21.4 (6)	+ 2.21	High calcium-phosphorus diet
7	11.35 (2) 11.17 (4)	3.59 (2) 3.02 (4)	6.75 (2) 6.73 (4)	41 (2) 33.7 (4)	+ 4.60	As above + 108 gm. $NaH_2PO_4.H_2O$
8	14.00 (3)	1.65 (3)	7.22 (3)	23 (3)	+ 2.66	Phosphate omitted
9	14.29 (1) 12.00 (4) 12.18 (6)	1.54 (1) 2.40 (4) 1.96 (6)	7.84 (1) 7.64 (4) 8.14 (6)	22 (1) 28.8 (4) 23.9 (6)	+ 3.36	As above + 52.8 gm. $NaH_2PO_4.H_2O$
10	12.44 (2)	1.63 (2)	6.67 (2)	20.3 (2)	+ 2.16	Phosphate omitted

Numbers in parenthesis show day of period on which sample of blood was taken. Values for first day of period were obtained prior to administration of sodium acid phosphate in that period.

+ = Calcium retained.

the patient remained essentially in nitrogen equilibrium. Calcium and phosphorus in the form of calcium lactate and calcium gluconate and acid sodium phosphate were now added to the ration in amounts sufficient to provide a level of intake comparable to that which had been given when the high calcium-phosphorus diet was ingested. Calcium was given at first as the lactate, but was discontinued and equivalent amounts of gluconate were substituted when it was found that calcium lactate produced nausea. Care was taken not to give the calcium and phosphorus salts together. The calcium was administered in divided doses and given with the meals. Phosphate was given four times daily and at least 2 hours before or after a meal. At this higher level of intake both elements were retained. Again the balances paralleled each other, but more phosphorus was retained than could have been utilized in the formation of $Ca_3(PO_4)_2$. With the omission of salts of calcium and phosphorus in the final control periods, the findings noted in the initial control periods were duplicated. Increasing the amounts of calcium and phosphorus in the ration (periods 22, 23 and 24) produced little change in the concentrations of Ca and inorganic P in the serum, yet the differences in urinary and fecal excretion of the two elements were of interest. The excretion of calcium in the urine was at the lowest level observed and the excretion in the feces which had previously been at an extraordinarily low level was markedly increased. The increased excretion of fecal calcium was probably due for the most part to calcium which had escaped absorption. While it is a temptation to speculate concerning possible reasons for the relatively poorer absorption of these salts (calcium lactate and gluconate) than of the calcium of the food, the data at hand are not sufficiently complete to permit conclusions to be drawn.

The partition of phosphorus between urine and feces remained essentially unaltered. The ratio, phosphorus in urine: phosphorus in feces was 5.5:1 in control periods and 5.9:1 at the higher level of intake of calcium and phosphorus. Throughout the entire experiment the excretion of phosphorus in the feces seems to have shown a tendency to parallel the amount of phosphorus in the ration and to have been fairly independent of the excretion of calcium in the feces. The latter observation is in agreement with the opinion expressed by Bulger and collaborators ('30, '31).

Magnesium. The nature of the bone lesions in hyperparathyroidism is such that one would expect to find the concentration of magnesium salts in bone reduced as are those of calcium. In a patient studied by Wilder ('29) the amount of magnesium in a specimen of bone removed at biopsy was normal, while the organic matter was increased and its content of calcium and phosphorus was definitely decreased. Bulger and Gausmann ('33), in a balance experiment, demonstrated a tendency for an individual with hyperparathyroidism to lose magnesium from the body. When a parathyroid tumor was removed, large amounts of calcium, phosphorus and magnesium were retained. No consistent changes in the concentration of magnesium in the blood were observed.

Data on the exchanges of magnesium in our patient are shown in figure 2 and table 2. Attention is called to the fact that, 1) approximate equilibrium between intake and outgo of magnesium was attained in periods 1 to 10 during which considerable amounts of calcium and phosphorus were stored; 2) that at a slightly higher level of magnesium intake (periods 11 to 19), there was retention of magnesium and, 3) that when the shift was made to the diet deficient in calcium (periods 19, 20 and 21), although there was coincidently a reduction in the intake of magnesium, no loss of magnesium from the body occurred. The exchanges of magnesium, therefore, showed considerable independence of the calcium in the food and of the calcium balance.

The administration of sodium acid phosphate affected the partition of magnesium between urine and feces. Large doses of phosphate produced a decrease in the excretion of magnesium in the urine (periods 7, 9, 14, 15 and 16) which was partly offset in some periods (9, 15 and 16) by an increased excretion of magnesium in the feces. The large fecal excretion of base (particularly calcium) in periods 22, 23 and 24 seems to have prevented an increase in the excretion of fecal magnesium (fig. 3). The quite definite decrease in urinary magnesium in these periods (presumably produced by phosphate) resulted in retention of magnesium. While these observations do not point to any characteristic abnormality in

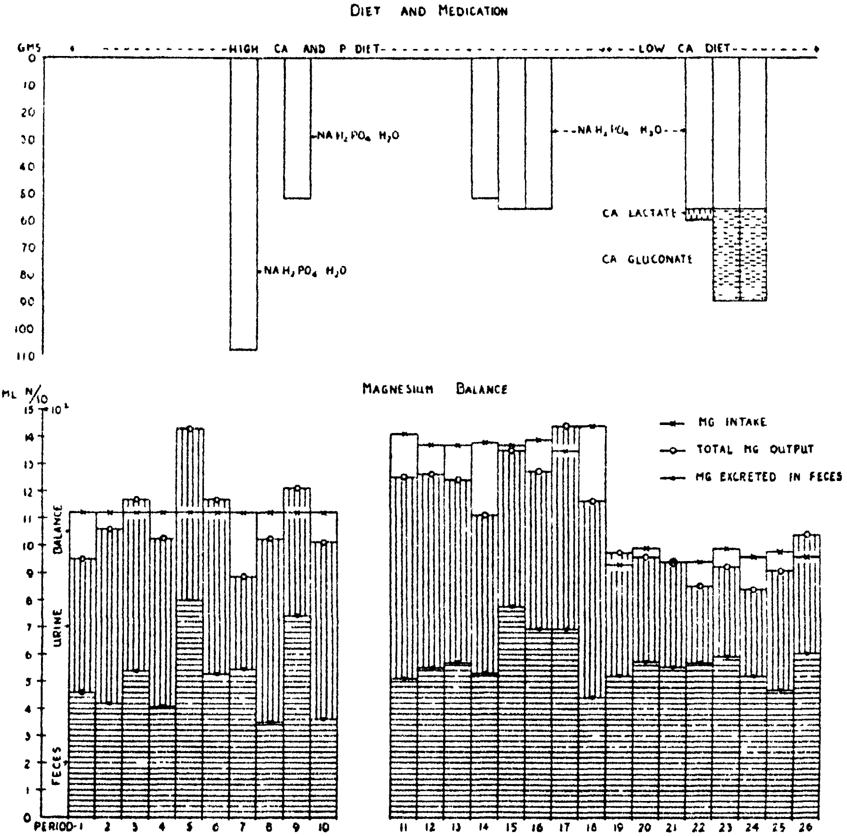

Fig. 2 Magnesium balance expressed as milliliter of N/10 solution (units of the scale × 10^2).

the partition of magnesium between urine and feces in hyperparathyroidism, nor to an excessive loss of this metal from the body during the course of the disease, we have subsequently been able to show that removal of a parathyroid tumor from the patient was followed by retention of considerable amounts of magnesium.

Sodium and potassium. Approximate equilibrium between intake and outgo of sodium and potassium was maintained throughout the experiment. Small positive and negative

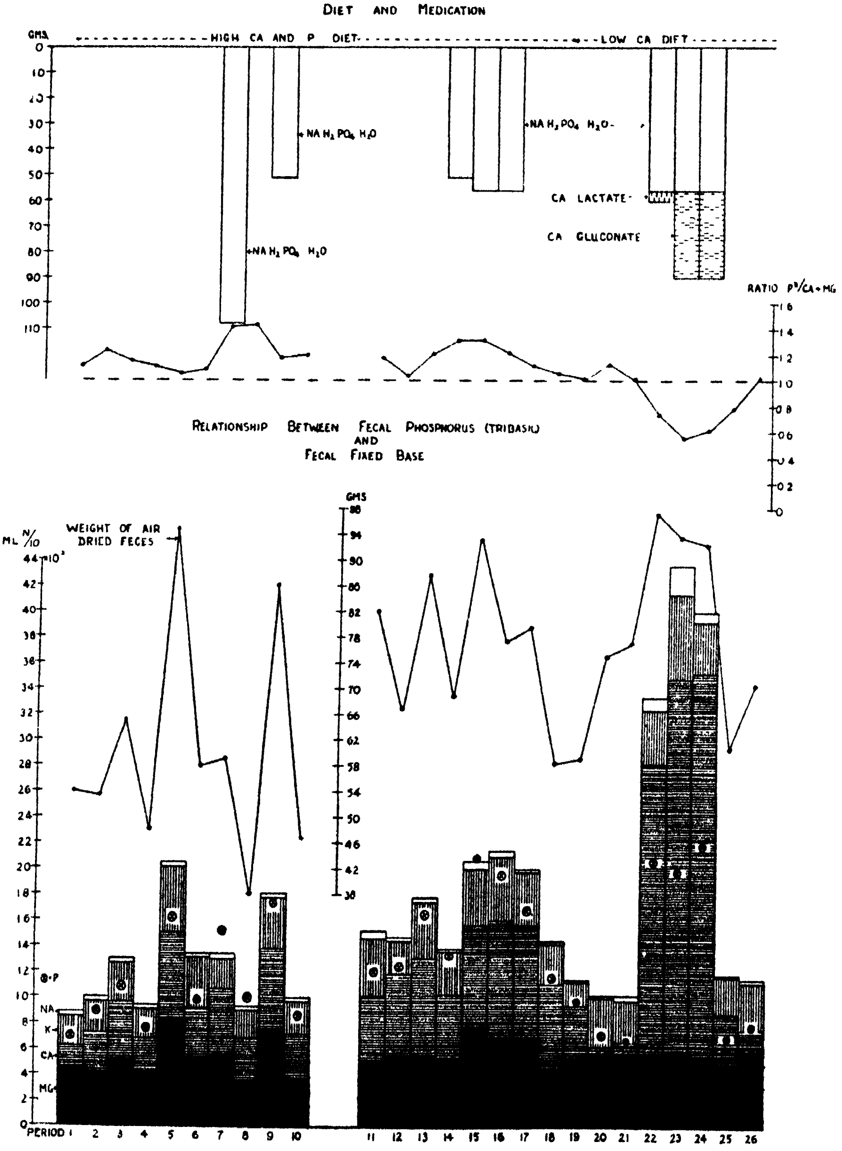

Fig. 3 Excretion of fixed base and phosphorus in the feces expressed as milliliter of N/10 solution. (Units of scale × 10^3). Phosphorus has arbitrarily been considered trivalent.

balances occurred in individual periods. Some degree of correlation was observed between balances of sodium (when calculated as extracellular water) and the fluctuations which occurred in body weight. A similar correlation between small retentions and losses of potassium and changes in the weight of the body could not be demonstrated.

TABLE 4

Effect of administration of sodium acid phosphate on sodium excretion in urine

PERIOD	SODIUM EXCRETION IN URINE ML. N/10	SODIUM EXCRETION IN EXCESS OF CONTROL ML. N/10	EXTRA SODIUM GIVEN WITH $NaH_2PO_4.H_2O$ ML. N/10	MEDICATION
Average of 12 and 13	[1] 7,560			None
14	11,890	4,330	3,869	$NaH_2PO_4.H_2O$ 52.29 gm.
15	11,780	4,220	4,056	$NaH_2PO_4.H_2O$ 55.96 gm.
16	12,525	4,965	4,043	$NaH_2PO_4.H_2O$ 55.82 gm.
17	7,510	—50		None
Totals for 14, 15, 16 and 17		13,465	11,959	
Average of 21 and 26	[1] 5,376			None
22	9,008	3,632	4,039	Calcium lactate 60 gm. $NaH_2PO_4.H_2O$ 55.78 gm.
23	9,504	4,128	4,074	Calcium gluconate 90 gm. $NaH_2PO_4.H_2O$ 56.23 gm.
24	8,839	3,463	4,087	Calcium gluconate 90 gm. $NaH_2PO_4.H_2O$ 56.40 gm.
25	6,026	650		None
Totals for 22, 23, 24 and 25		11,873	12,200	

[1] Control periods: periods 12 to 17, inclusive, high calcium diet; periods 21 to 25, inclusive, low calcium diet.

The excretion of potassium was not affected by the ingestion of as much as 18 gm. of sodium acid phosphate per diem for 6 days.

Extra sodium administered as the acid phosphate was almost quantitatively excreted in the urine (table 4) either during the period in which it was ingested or during the first after period.

Fecal sodium was not materially increased during periods of ingestion of phosphate (fig. 3), although, as previously stated, the absolute amount of fecal phosphorus was considerably increased. We have found that a normal subject, while ingesting a similar diet, failed to show an increased excretion of sodium in the feces even when the amount of fecal phosphorus was more than doubled by administration of sodium acid phosphate. This seems to disprove the contention of Salter, Farquharson and Tibbetts ('32) that the administration of sodium orthophosphate leads to the excretion of considerable amounts of disodium phosphate in the feces.

Balance of acid and base in urine. The data on excretion of acid and base in the urine are represented graphically in figure 4. Since organic acids and bicarbonate were not actually determined, it has been assumed that they were equal to the difference between the sum of the basic components and the total excretion of inorganic acids. No distinctive features were noted in control periods other than the unusually large excretion of calcium and phosphorus. The changes in urinary acid and base produced by the administration of $NaH_2PO_4.H_2O$ were similar to, if not identical with, those described by Farquharson, Salter and Aub ('31) in urine of normal subjects who ingested acid phosphate. In brief, it may be stated that in uncomplicated hyperparathyroidism the excretion of acid and base in the urine conforms closely to the normal pattern. This substantiates the work of Albright, Bauer and Aub ('31).

DISCUSSION

Sodium and potassium. Greenwald ('29) has suggested that the retention of base (sodium and potassium) which follows parathyroidectomy may be due to a disturbance of renal function brought about by the lowered concentration of calcium in the serum. Experiments of Albright and Ellsworth ('29) and Ellsworth ('32) strongly suggest that parathormone has an effect on renal function, since a diuresis of phosphorus and lowering of the concentration of inorganic phosphorus in the serum promptly follow injection of the hormone. These ef-

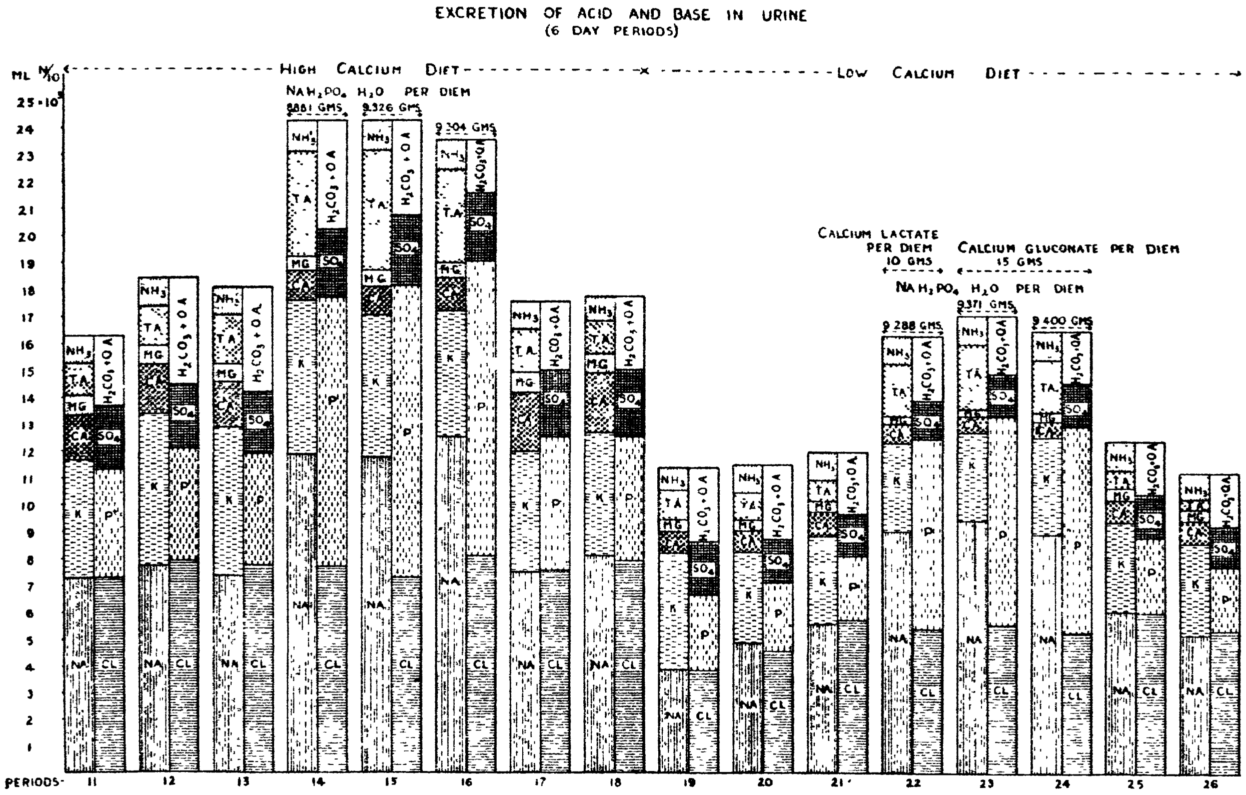

Fig. 4 Acid-base balance in urine expressed as milliliter of N/10 solution. (Units of scale × 10³.) The organic acid (O.A.) and bicarbonate have been estimated as the difference between the sum of the cations, which include ammonia and titratable acid, and the sum of the anions Cl, P and SO_4.

fects were noted prior to an increase in the concentration of calcium in the serum and hence the latter is presumably not responsible for alteration of kidney function. The results of their experiments have led them to postulate a lowered renal threshold for phosphorus as perhaps the most fundamental effect of the parathyroid hormone. Further evidence that the disturbance in the equilibrium of electrolytes produced by excessive excretion of phosphorus and calcium is accompanied by changes in the balances of other inorganic salts has been furnished by Albright, Bauer and Aub ('31). They produced hyperparathyroidism in a patient with myositis ossificans by injections of parathormone and observed some increase in the excretion of water, base (in addition to calcium) and chloride. A reversal of these phenomen occurred when the injections of the hormone were discontinued. In so far as the present study of uncomplicated hyperparathyroidism is concerned, we were unable to demonstrate any tendency toward excessive excretion or retention of sodium, potassium or chlorine, nor were there any alterations in the normal partition of these elements between urine and feces. Our results are not incompatible with the observations of Albright and collaborators, since the effects that these investigators describe appear to have been of a temporary nature. Noteworthy changes in the balances of water and electrolytes (exclusive of calcium and phosphorus) are probably to be expected only at times when there is a sudden increase in the production of parathormone or when the production of the parathyroid secretion is suddenly decreased or stopped as in the case of removal of a parathyroid tumor or parathyroidectomy.

Calcium and phosphorus. In the light of more complete knowledge of the ultimate consequences of the hyperparathyroid state, the advisability of treating this disease with high calcium diets and acid phosphate is open to serious question. Recent papers by Bauer ('33) and by Albright, Baird, Cope and Bloomberg ('34) show quite clearly the futility of treatment in some cases and the complications which may arise in others when attempts are made to prevent further decalcification of the skeleton or to cause healing of the bone lesions by ingestion of a high calcium diet. While it is admitted that suitable dietary measures can cause deposition of calcium salts in the bones of the milder cases of hyperparathyroidism, Albright and collaborators ('34) report the development of considerable impairment of renal function in such a patient who had been treated by the administration of a high calcium diet. They show evidence which leads to the conclusion that the renal injury was produced by the formation of pathological calcifications in the tissues of the kidney or within the renal tubules. That conditions which favor the formation of these deposits may well be produced or enhanced by the ingestion of a high calcium diet is shown admirably in the case of our patient. During control periods in which she received food rich in calcium, both the quantity and con-

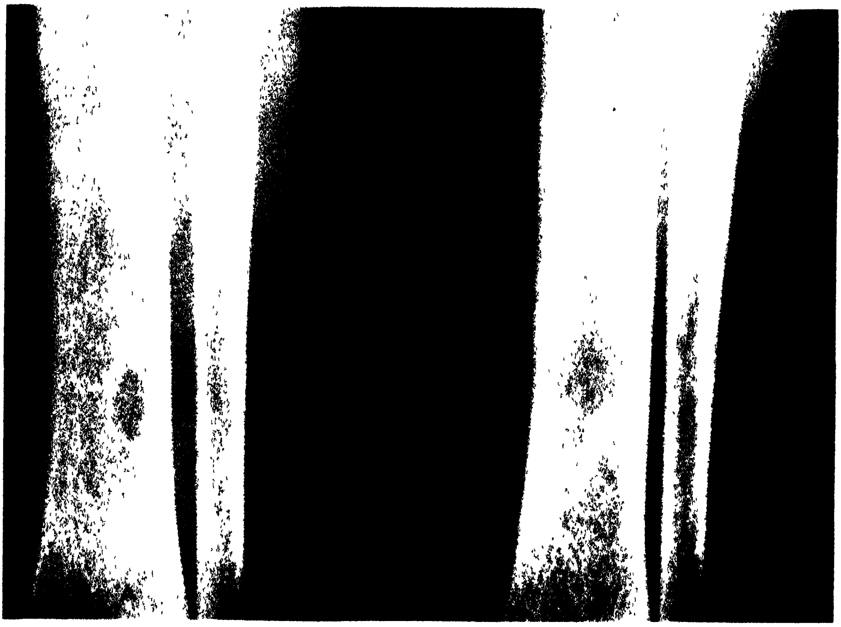

Fig. 5 A) Roentgenogram of shaft of left tibia prior to administration of a high calcium phosphorus diet. B) Shaft of right tibia after 3 months on high calcium phosphorus diet, showing calcification of cyst.

centration of calcium in the urine was two- to threefold greater than in similar control periods in which she ingested food poor in calcium. Fortunately, the urea clearance test (Van Slyke) (Peters and Van Slyke, '31) and creatinine filtration rate (Rehberg) (Holton and Rehberg, '31) showed no impairment of renal function after the patient had ingested a high calcium diet for nearly a year. At the conclusion of this period of treatment, one bone cyst had recalcified (fig. 5). However, a roentgenogram showing the proximal end of the shaft of the same bone disclosed the formation of a new cystic area which had apparently developed simultaneously with the healing of the cyst in the more distal portion of the shaft. Furthermore, metabolic data suggest that the immediate storage of calcium and phosphorus in the body which followed the institution of a high calcium phosphorus regimen was not well sustained. This is illustrated by the fairly rapid and apparently progressive decrease in the positive balances of these elements which took place in consecutive control periods.

The evidence cited, together with the fact that there was no indication, after more than a year of observation, of abatement in the severity of the metabolic disorder, led to a decision to explore the region of the thyroid in search of a parathyroid tumor. Metabolic studies carried on at the time the tumor was discovered and removed are reported separately.

SUMMARY

1. The ingestion of a diet rich in calcium and phosphorus by a patient with mild hyperparathyroidism resulted in retention of these elements in approximately the proportions in which they occur in bone. During 108 days the total amount of calcium retained was 60 gm., of phosphorus 34.7 gm. and of nitrogen 70 gm. In spite of this, the ingestion of a high calcium diet for 1 year did not result in appreciable recalcification of the skeletal lesions.

2. The daily administration of from 10 to 18 gm. of sodium acid phosphate increased the concentration of inorganic phosphorus in blood plasma and decreased hypercalcemia and

hypercalcinuria. The amount of calcium and phosphorus retained was increased during the periods in which acid phosphate was ingested. These effects were temporary and ceased as soon as the administration of phosphate was discontinued.

3. Calcium of the food was much more completely absorbed than calcium administered in the form of lactate or gluconate.
4. The ingestion of a low calcium diet was attended by a marked decrease in the amount of calcium excreted in the urine.
5. The exchanges of magnesium were not materially influenced by the state of the calcium balance.
6. The ingestion of large amounts of sodium acid phosphate caused a decrease in the amount of magnesium excreted in the urine and in several instances an increase in the excretion of magnesium in the feces.
7. Extra phosphoric acid, administered as sodium acid phosphate, was excreted chiefly in the urine. Provision was made for the elimination of this excess of acid by, a) increase in the titratable acid in the urine, b) decrease in the excretion of undetermined acid in the urine (bicarbonate plus organic acid) and, c) by an increase in excretion of fixed base (sodium) in amounts equivalent to those present in the sodium acid phosphate which was ingested.
8. The excretion of fecal phosphorus showed a slight increase as a result of administration of phosphate, and there was usually a concomitant increase in the excretion of fecal fixed base in the form of calcium and magnesium. There was little or no correlation between excretion of phosphorus in the feces and the fecal excretion of sodium and potassuim.
9. Sodium ingested with the food, as a solution of sodium chloride and as sodium acid phosphate was eliminated almost quantitatively in the urine.
10. Equilibrium between intake and outgo of potassium was maintained throughout the period of observation.

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MINERAL EXCHANGES OF MAN

VI. THE EFFECT OF EXTIRPATION OF A PARATHYROID TUMOR ON THE BALANCES OF ELECTROLYTES

SAMUEL H. BASSETT AND HELEN E. VAN ALSTINE[1]
Department of Medicine, University of Rochester School of Medicine and Dentistry and Medical Clinic of Strong Memorial Hospital, Rochester, New York

FOUR FIGURES

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Metabolic experiments performed by Greenwald ('13) and Greenwald and Gross ('25) provided the first satisfactory evidence of the effect of parathyroidectomy on the excretion of calcium and phosphorus. That retention of these elements follows the removal of the parathyroid glands has been confirmed repeatedly, but it is less clearly established that this operation is capable of influencing the metabolism of other inorganic constituents of the body.

In one of his earlier experiments Greenwald ('13) noted that primary retention of phosphorus was followed by a secondary decrease in the excretion of sodium and potassium in the urine. He confirmed this observation later (Greenwald, '29) and suggested by way of explanation that both "the increased concentration of inorganic phosphorus in the serum and the diminished excretion of sodium and potassium" might be due to an "interference with renal function brought about by the lowered calcium concentration in the plasma."

Although removal of a parathyroid tumor for therapeutic purposes seems to have many points in common with experimental parathyroidectomy in animals (Bulger, Dixon, Barr

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and Schregardus, '30) even on occasion being followed by severe tetany (Bulger et al., loc. cit.; Snapper, '30 and others) the question of its effect on the metabolism of sodium and potassium does not appear to have been investigated. Evidence that the parathyroid hormone has some action on the mechanisms which regulate the volume of body water in man is not altogether lacking. A diuretic action has been attributed to parathormone by several of those who have investigated its therapeutic and physiological potentialities (Davidson, '25; Mason, '26; McCann, '28; Johnson, '31). A metabolic study by Albright, Bauer and Aub ('31) showed that when hyperparathyroidism was produced by the injection of parathormone, a small increase in the excretion of water, chloride and base followed the initial injections, with subsequent retention of these elements when the treatment was discontinued. They stated that no evidence was obtained which indicated a fundamental disturbance in the acid-base balance of the body. We have previously given attention to this phase of metabolism without being able to detect losses of sodium or potassium during the hyperparathyroid state (Bassett, '35). While such losses if small might easily have been obscured by errors in experimental technic, it seems more probable, as the data of Albright and co-workers suggest, that changes in the volume of water and its solutes may be limited to short periods of readjustment, which are initiated by sudden increases or decreases in the amount of parathormone made available to the organism. In order to subject this hypothesis to further test, we have followed the exchanges of sodium, potassium, calcium, magnesium, chlorine and phosphorus both before and during the critical period which follows removal of a parathyroid tumor.

EXPERIMENTAL PROCEDURE AND METHODS

The same patient, A. L. no. 48736, who served as the subject of the former investigation and whose clinical history has been reported in detail by Morton ('33), re-entered the metabolism unit of the hospital for the necessary pre-operative studies.

The mineral exchanges were followed for 21 days prior to extirpation of a parathyroid tumor and for 27 days subsequent to the operation. The patient remained in the metabolism unit throughout this time, except for a brief interval of approximately 3 hours which was spent in the operating room. The operation was accomplished with practically no loss of blood and with but slight manipulation of the thyroid apparatus. There was no visible sweating during operation nor in the period of recovery from the anaesthetic (avertin and nitrous oxide). Following operation, the patient vomited once. The amount of vomitus did not exceed 100 cc. and was added to the excreta for analysis. All collections of urine and feces were quantitative. For 3 days preceding operation the urine was saved in 12-hour day and night specimens and each of these was analyzed for sodium, potassium, calcium, chlorine and phosphorus. The same procedure was followed during the first 6 days of convalescence, except in one or two instances when, because of difficulty in voiding, the collection periods exceeded 12 hours. Other than this collections of excreta were made for the usual metabolic period of 3 or 6 days. Daily analyses of urine were then restricted to the determination of ammonia, titratable acidity minus carbonates (Albright and Bauer, '29), total organic acids (Van Slyke and Palmer, '20) and creatinine. Representative samples of each serving of food were saved, pooled, and analyzed to determine the intake of mineral elements in each period (Bassett and Van Alstine, '35). Table 1 gives the average daily intake of carbohydrate, protein, fat, calories, water, calcium and phosphorus for each metabolic period. The alterations in the level of intake in periods 31 and 32 were unavoidable, since the patient experienced some difficulty in swallowing during early convalescence and could not at once resume the diet of the fore periods.

Favorable experimental and clinical reports on the use of irradiated ergosterol for relief of parathyreoprevic tetany (Wade, '29; Brougher, '30; Bauer, Marble and Claflin, '32; Schultzer, '32) led to its use as a prophylactic measure. Approximately 3000 rat units of viosterol (Squibb) were given daily for 3 days preceding removal of the tumor and the dose was increased to 3750 units on the second post-operative day when mild tetany appeared. One intravenous injection of 50 units of parathormone was also given on this day. Tetany did not appear again in an active form, although there were occasional signs of latent tetany until the date of discharge from the hospital. Viosterol was continued through the eleventh post-operative day and then omitted.

TABLE 1

Average daily intake of protein, carbohydrate, fat, water, calories, calcium and phosphorus

PERIODS	PROTEIN	CHO	FAT	WATER[1]	CALORIES	CALCIUM	PHOSPHORUS	RATIO Ca/P	COMMENT
	gm.	*gm.*	*gm.*	*Liters*		*gm.*	*gm.*		
27, 28, 29, 30, 33, 34, 35	81	243	106	3.36	2314	1.127	1.543	0.73	21 days before operation and 9th to 26th day, inclusive, after operation
31[2]	62	203	91	3.26	1933	1.471	1.338	1.10	First 5 days after operation
32	63	142	83	2.9	1612	1.133	1.286	0.88	6th to 8th days, inclusive, after operation

[1] Water drunk and water of food.

[2] Day of operation not included, since no food was given on that day.

On the day of operation 1500 ml. of salt solution containing 0.7 per cent of sodium chloride, 0.03 per cent of potassium chloride and 0.025 per cent of calcium chloride were administered subcutaneously. This comprised practically the entire salt intake for the day. Water was given by mouth in small amounts after consciousness had been regained. The intake of fluid was thus brought to a total of 2350 ml. Thereafter liquid foods and water were ingested in moderate quantities (average daily values appear in table 1) until the sixth post-operative day when a semi-solid diet was again tolerated.

METABOLIC DATA

The actual balances which were computed for each period, exclusive of the acid-base balance of the urine, are shown in tables 2 and 3. For purposes of discussion, it will be convenient to point out the major changes produced by the removal of the parathyroid tumor and then proceed with a more detailed examination of metabolic effects in the case of single elements or groups of closely related elements.

After about 18 days of nearly constant intake of food and water and of carefully regulated activity, approximate equilibrium between intake and outgo of mineral elements was obtained. The parathyroid tumor was then excised. An immediate retention of phosphorus, base, chlorine and water followed this procedure. The remarkable daily retention of base in the early stages of recovery is seen in figure 1.

Calcium and phosphorus. The changes in the metabolism of calcium and phosphorus were most striking, but are now so well known that they require but little comment (Bulger et al., '30; Hunter and Turnbull, '31). Our findings conformed closely to those published in the literature. There was a precipitate fall in serum calcium. Within 24 hours after operation, it had declined from 12.5 to 9.2 mg. per cent. The post-operative excretion of phosphorus in the urine practically ceased for 36 hours. The excretion of calcium in the urine decreased somewhat less abruptly, but continued to decline gradually until it became established at about one-fifth of its pre-operative level. The excretion of urinary phosphorus, on the other hand, began to increase after the second post-operative day, but did not regain its former high level of excretion. Little change occurred in the concentration of inorganic phosphorus in the serum until about the time at which the urinary excretion of phosphorus became reestablished, when it increased from 2 to approximately 3 mg. per cent. The increase in the concentration of inorganic phosphorus in the serum and the increased excretion of this element in the urine, therefore, coincided almost perfectly.

TABLE 2

Balances of nitrogen, calcium, magnesium and phosphorus

PERIOD	NITROGEN, GM.			CALCIUM, GM.			MAGNESIUM, GM.			PHOSPHORUS, GM.			COMMENT
	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
27 6 days Jan. 9–14	Total 75.1	U 63.3 F. 4.9 68.2	+ 6.9	6.56	U. 3.55 F. 1.22 4.77	+ 1.79	2.148	U. 0.811 F. 0.933 1.744	+ 0.404	9.17	U. 5.90 F. 1.29 7.19	+ 1.98	Control periods
28 6 days Jan. 15–20	Total 75.0	U. 62.6 F. 4.9 67.5	+ 7.5	6.62	U. 3.28 F. 2.01 5.29	+ 1.33	2.136	U. 0.842 F. 1.236 2.078	+ 0.058	9.28	U. 6.69 F. 1.66 8.35	+ 0.93	Control periods
29 6 days Jan. 21–26	Total 71.6	U. 62.5 F. 5.0 67.5	+ 4.1	6.78	U. 3.32 F. 2.54 5.86	+ 0.92	2.148	U. 0.787 F. 1.277 2.064	+ 0.084	9.24	U. 6.69 F. 1.67 8.36	+ 0.88	Control periods
30 3 days Jan. 27–29	Total 36.4	U. 33.3 F. 3.5 36.8	— 0.4	3.35	U. 1.71 F. 2.01 3.72	— 0.37	1.040	U. 0.437 F. 0.857 1.294	— 0.254	4.58	U. 3.37 F. 1.33 4.70	— 0.12	Control periods
Totals for 21 days	Total 258.1	240.0	+ 18.1	23.31	19.64	+ 3.67	7.472	7.180	+ 0.292	32.27	28.60	+ 3.67	Totals pre-operative periods
31 6 days Jan. 30–Feb. 4	Total 49.7	U. 42.3 F. 3.2 45.5	+ 4.2	8.83	U. 1.02 F. 1.86 2.88	+ 5.95	1.332	U. 0.442 F. 0.645 1.087	+ 0.245	8.03	U. 2.05 F. 1.05 3.10	+ 4.93	Operation January 30 liquid diet
32 3 days Feb. 5–7	Total 30.1	U. 31.1 F. 1.6 32.7	— 2.6	3.40	U. 0.16 F. 0.93 1.09	+ 2.31	0.571	U. 0.206 F. 0.322 0.528	+ 0.043	3.86	U. 2.52 F. 0.53 3.05	+ 0.81	Semi-solid diet
33 6 days Feb. 8–13	Total 78.7	U. 58.5 F. 4.5 63.0	+ 15.7	7.03	U. 0.35 F. 1.54 1.89	+ 5.14	2.116	U. 0.614 F. 1.221 1.835	+ 0.281	9.63	U. 4.59 F. 1.60 6.19	+ 3.44	Pre-operative diet
34 6 days Feb. 14–19	Total 76.5	U. 50.9 F. 5.7 56.6	+ 19.9	6.87	U. 0.34 F. 1.19 1.53	+ 5.34	2.000	U. 0.548 F. 1.064 1.612	+ 0.388	9.14	U. 4.11 F. 1.46 5.57	+ 3.57	Pre-operative diet
35 6 days Feb. 20–25	Total 74.9	U. 61.1 F. 5.7 66.8	+ 8.1	6.76	U. 0.28 F. 1.62 1.90	+ 4.86	1.976	U. 0.671 F. 1.172 1.843	+ 0.133	9.16	U. 4.98 F. 1.59 6.57	+ 2.59	Pre-operative diet
Totals for 27 days	Total 309.9	264.6	+ 45.3	32.89	9.29	+ 23.60	7.995	6.905	+ 1.090	39.82	24.48	+ 15.34	Totals for post-operative periods

Legend: U = excretion in urine; F = excretion in feces; + = retention; — = loss.

TABLE 3

Balances of sodium, potassium and chlorine

PERIOD	WEIGHT,[1] KG.	SODIUM, GM.			POTASSIUM, GM.			CHLORINE, GM.			COMMENT
		Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
27	51.010	NaCl 7.08	U. 12.52			U. 25.10		10.92	U. 21.60		Control periods
6 days	(+ 570)	Food 6.08	F. 0.04			F. 1.66		11.44	F. 0.09		
Jan. 9–14		Total 13.16	12.56	+ 0.60	29.00	26.76	+ 2.24	22.36	21.69	+ 0.67	
28	51.580	NaCl 7.08	U. 10.15			U. 22.69		10.92	U. 19.32		Control periods
6 days	(+ 820)	Food 5.49	F. 0.04			F. 1.85		10.68	F. 0.12		
Jan. 15–20		Total 12.57	10.19	+ 2.38	28.16	24.54	+ 3.62	21.60	19.44	+ 2.16	
29	52.400	NaCl 7.08	U. 12.78			U. 25.85		10.92	U. 21.31		Control periods
6 days	(+ 20)	Food 5.49	F. 0.06			F. 1.91		11.53	F. 0.21		
Jan. 21–26		Total 12.57	12.84	— 0.27	27.96	27.76	+ 0.20	22.45	21.52	+ 0.93	
30	52.420	NaCl 3.54	U. 6.98			U. 12.63		5.46	U. 12.21		Control periods
3 days	(— 80)	Food 2.81	F. 0.45			F. 1.98		5.30	F. 0.65		
Jan. 27–29		Total 6.35	7.43	— 1.08	14.22	14.61	— 0.39	10.76	12.86	— 2.10	
Totals for 21 days		44.65	43.02	+ 1.63	99.34	93.67	+ 5.67	77.17	75.51	+ 1.66	Totals for pre-operative periods
31	52.340	NaCl 3.53	U. 5.26			U. 9.60		6.77	U. 11.53		Operation January 30 liquid diet
6 days	(+ 1310)	Food 6.61	F. 0.12			F. 1.77		11.91	F. 0.52		
Jan. 30–Feb. 4		Total 10.14	5.38	+ 4.76	17.36	11.37	+ 5.99	18.68	12.05	+ 6.63	
32	53.650	NaCl 3.54	U. 5.17			U 9.58		5.46	U. 7.66		Semi-solid diet
3 days	(— 770)	Food 2.92	F. 0.06			F. 0.89		5.03	F. 0.26		
Feb. 5–7		Total 6.46	5.23	+ 1.23	7.92	10.47	— 2.55	10.49	7.92	+ 2.57	
33	52.880	NaCl 7.08	U. 10.24			U. 24.90		10.92	U. 19.22		Pre-operative diet
6 days	(+ 1220)	Food 5.54	F. 0.21			F. 1.80		10.40	F. 0.23		
Feb. 8–13		Total 12.62	10.45	+ 2.17	28.92	26.70	+ 2.22	21.32	19.45	+ 1.87	
34	54.100	NaCl 6.69	U. 12.36			U. 21.45		10.32	U. 21.91		Pre-operative diet
6 days	(— 560)	Food 5.20	F. 0.39			F. 2.43		10.71	F. 0.26		
Feb. 14–19		Total 11.89	12.75	— 0.86	27.08	23.88	+ 3.20	21.03	22.17	— 1.14	
35	53.540	NaCl 7.08	U. 11.39			U. 25.30		10.92	U. 20.87		Pre-operative diet
6 days	(+ 440)	Food 5.88	F. 0.44			F. 2.88		12.20	F. 0.34		
Feb. 20–25	53.980	Total 12.96	11.83	+ 1.13	28.12	28.13	— 0.01	23.12	21.21	+ 1.91	
Totals for 27 days		54.07	45.64	8.43	109.40	100.55	+ 8.85	94.64	82.80	+ 11.84	Totals for post-operative periods

[1] Weights are given for the first day of each metabolic period, except in period 35 where the final weight recorded is for the close of this period. Values in parenthesis give the gain or loss of weight in grams which occurred in the period.

Legend: U = excretion in urine; F = excretion in feces; + = retention; — = loss.

When the moderately high intake of calcium and phosphorus is considered, the excretion of each in the feces during the pre-operative periods is seen to have been distinctly less than normal. Following the operation, there was little or no change in the the amount of fecal phosphorus, while the excretion of calcium in the feces actually appeared to decrease slightly.

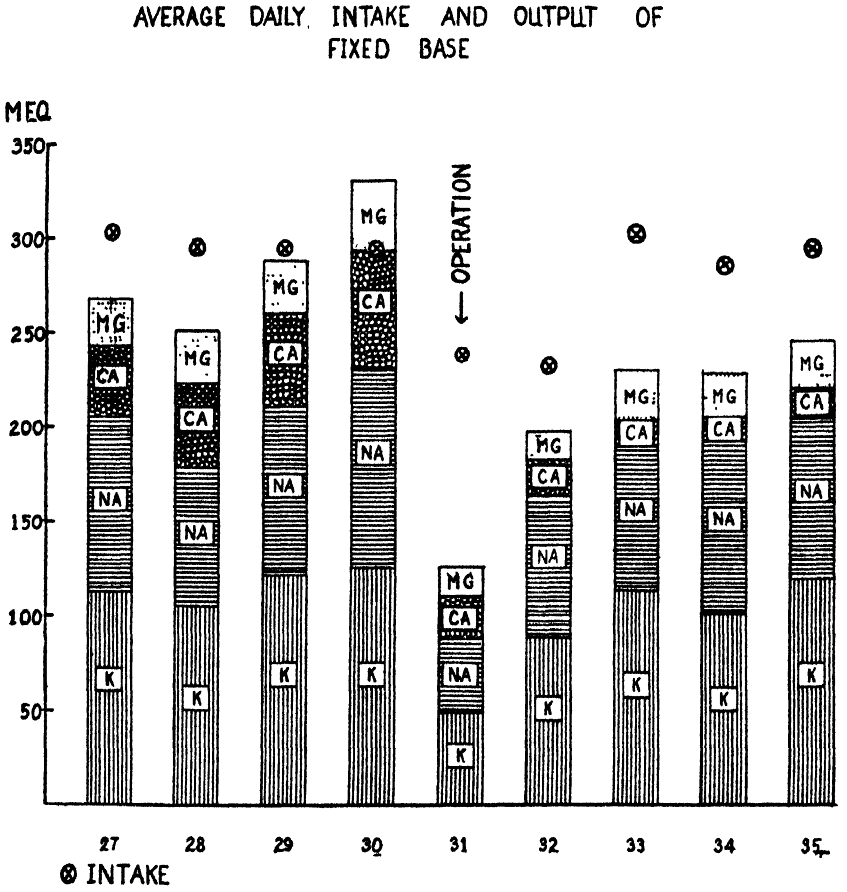


Fig. 1 The height of each column represents the total excretion of fixed base. Individual components are indicated by the appropriate chemical symbol. 'Intake' refers to the total amount amount of fixed base furnished by diet and solution of sodium chloride. Numerals at the foot of each column show the period for which the average has been computed. Operation was performed on the first day of period 31.

A very marked retention of both calcium and phosphorus occurred post-operatively and continued until the experiment was terminated. The total retentions were roughly in the proportions in which these elements occur in bone. However, this was not necessarily the case in individual periods. For example, much more phosphorus was retained in the operative period (period 31) than was needed to combine with the calcium simultaneously retained. Attention has again been directed to this fact in the subsequent discussion of the metabolism of sodium and potassium.

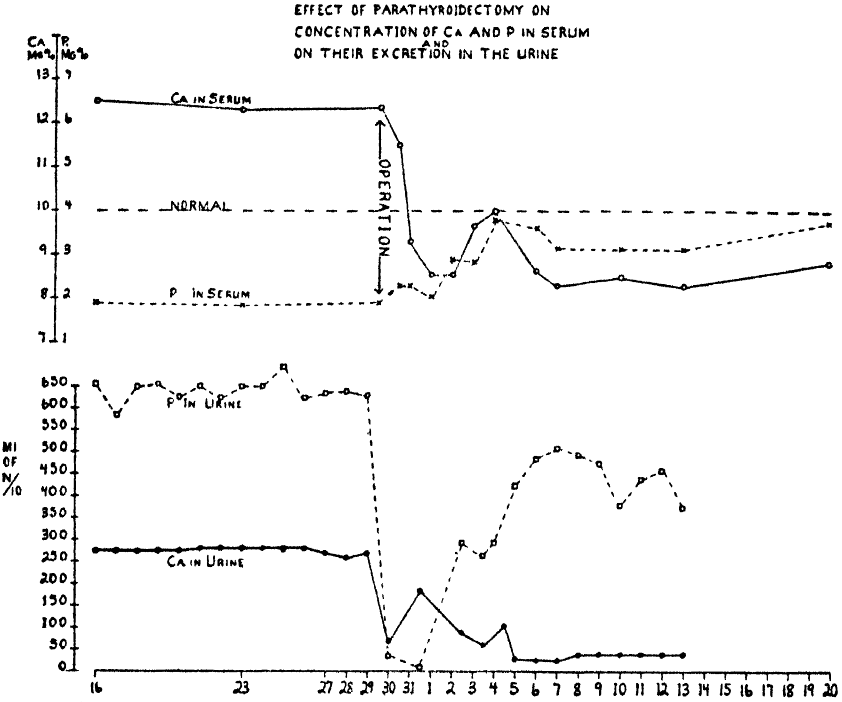


Fig. 2 The immediate effects of removal of the parathyroid tumor on calcium and phosphorus of blood and urine are shown in the chart. Note the almost complete disappearance of phosphorus from the urine without significant increase in the concentration of inorganic phosphorus in the serum.

Magnesium. The effect of parathyroidectomy (removal of a parathyroid tumor) on the metabolism of magnesium appears to be of the same nature as its effect on the metabolism of calcium and phosphorus. Distinctly more magnesium was retained following operation than before it, and the excretion of magnesium in the urine was moderately decreased in post-operative periods. Our observations, therefore, confirm those reported by Bulger and Gausmann ('33).

Nitrogen. Equilibrium, which appears to have been established prior to operation, was maintained during the first 9 days of convalescence. Thereafter with return to the more liberal diet provided in the pre-operative periods, there was definite retention of nitrogen. Such nitrogen retention seems logical during a period of clinical improvement of the patient, especially since weakness and hypotonia of the muscles are frequent manifestations of the hyperparathyroid state.

Sodium, potassium and chlorine. Immediately after removal of the parathyroid tumor, the excretion of sodium, potassium and chlorine in the urine became markedly reduced (fig. 3). The data show that most of the salt administered subcutaneously and immediately after operation was retained in the body, and that the excretion of sodium did not become equal to the amount ingested until the fifth post-operative day. The excretion of chlorine was in most instances equivalent to that of sodium and hence has been omitted from the graph. The net sodium and chlorine retentions for period 31 were: sodium 207 milli-equivalents (m.eq.); chlorine 187 m.eq.

The curve of the excretion of potassium also followed a course almost identical to that of sodium during early convalescence. Since very little potassium was given on the day of operation, a small loss of potassium occurred on this day. However, on subsequent days with the level of potassium intake reduced somewhat below the preoperative level, there was marked retention of potassium. The net balance for the period (period 31) showed a retention of 152 m.eq. of potassium. Whether the retention of potassium in period 31 was related in any manner to the apparently excessive retention of phosphorus in this period is a matter for conjecture. Should all of the calcium deposited in the period have been in the form of tricalcium phosphate, there yet remained 108 m.eq. of

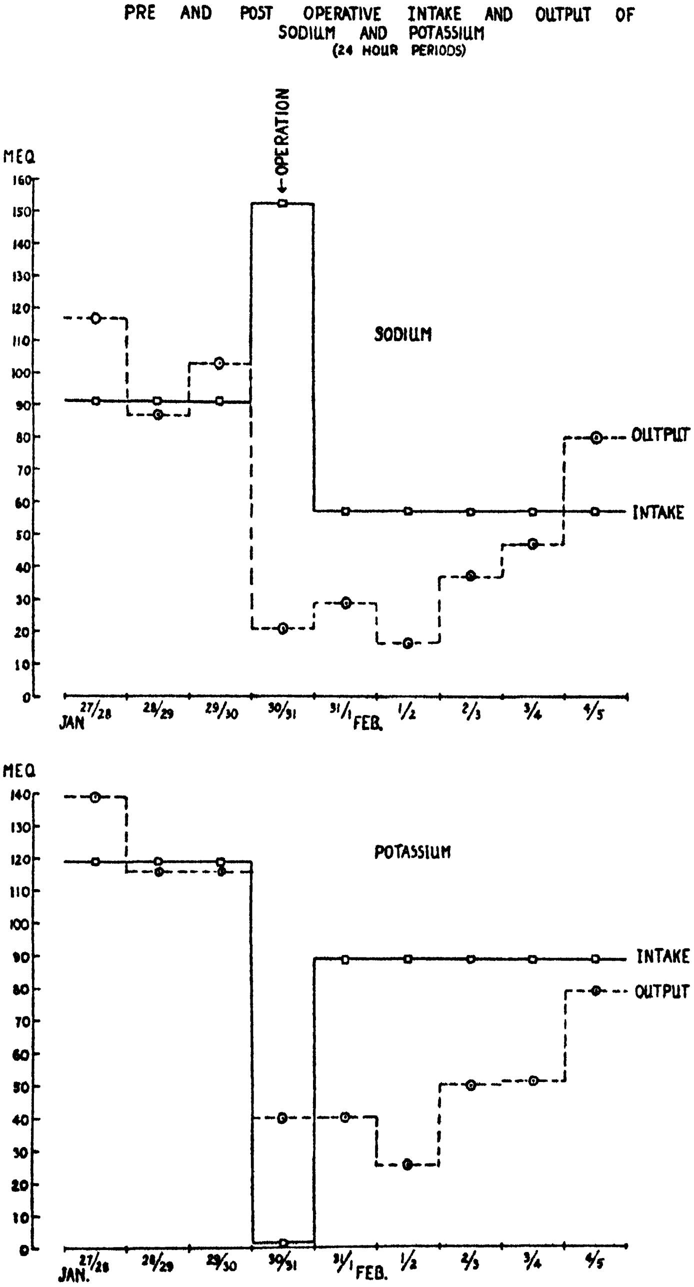
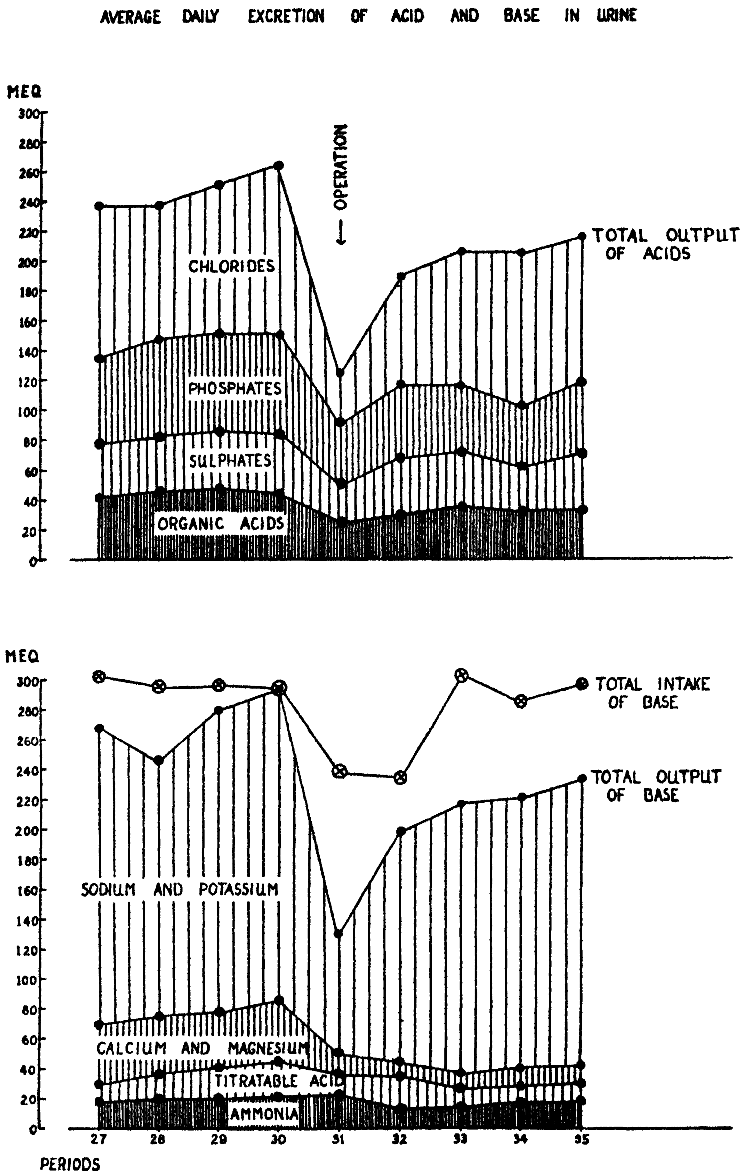


Fig. 3 Operation was performed on the morning of January 30th. Note retention of sodium and potassium on this and subsequent days.



AVERAGE DAILY EXCRETION OF ACID AND BASE IN URINE

Fig. 4 The acid-base balance of the urine has been computed and illustrated in the manner suggested by Gamble, Ross and Tisdall ('23), except that the urinary excretion of carbonates has not been estimated. The very marked decrease in the excretion of acid and base in the urine during period 31 was in part due to decreased intake, and in part due to retention of calcium, magnesium, sodium, potassium, phosphorus and chlorine in the body.

phosphorus (calculated at a valence of 1.8) which might have been neutralized by potassium. As implied in the introductory remarks, this suggestion was brought forward by Greenwald ('13) to explain the retention of base (other than calcium) which followed the operation of thyreoparathyroidectomy in dogs.

Excretion of acid and base in the urine. The average daily excretion of acid and base in the urine in each period is shown in figure 4. The data have been arranged in such a manner that the areas between lines are roughly proportional to the several components comprising the total excretion. The factors which conserve fixed base (ammonia and titratable acid) have been included in the diagram which represents the total excretion of base (Gamble, Ross and Tisdall, '23) and hence the latter should exactly balance the excretion of acid. This appears to be the case within limitations imposed by methods.

The major changes occurred in period 31 and these, as noted above, followed in part as the result of decreased intake of mineral elements in the food and in part because of marked retention of fixed base, chlorine and phosphorus. With return to the preoperative diet (periods 33, 34 and 35), aside from decreased excretion of calcium, phosphorus, and magnesium, there was noted a very small decrease in the excretion of ammonia, a somewhat greater decrease in the excretion of titratable acid and a slight decrease in the excretion of organic acid. The decrease in the excretion of ammonia and titratable acid was probably due to diminished excretion of phosphates and organic acids.

DISCUSSION

We are of the opinion that the moderate and apparently temporary disturbance of salt and water metabolism which has been depicted for the operative period was part of a general readjustment of electrolyte balance made necessary by the removal of the parathyroid tumor. This position is difficult to maintain, unless it can be shown that the general effects of the operative procedure and of the unavoidable departures from the routine of the fore periods had no significant relation to the results obtained or were actually inimical to them. Perhaps the most obvious fallacy in the usual method of estimating the balances of sodium and chlorine is the well-known fact that variable amounts of salt may be excreted in sweat (Cramer, 1890; Atchley et al., '33) and that such losses remain unrecorded. Moreover, loss of water and electrolytes through bleeding introduces another source of error which should not be disregarded (Coller and Maddock, '32). It is impossible to answer the first objection in an entirely satisfactory manner without measurement of excretion by the skin. Observations have, however, been made of the concentration of sodium in sweat (Bassett and Warren, '33) under conditions which undoubtedly place this value too high. A simple computation based on these data shows that one would be forced to assume a secretion of about 1100 ml. of sweat on the day of operation if the apparent retention of 132 m.eq. of sodium during the 24 hours involved were due entirely to skin losses. It is extremely improbable that such an amount of sweating could have occurred and yet have remained undetected. Bleeding as a source of error can be excluded with certainty, since excellent hemostasis was obtained.

The effect of anesthesia (avertin plus nitrous oxide and oxygen) is less readily evaluated, but presumably favors dehydration (Coller and Maddock, '32) and hence loss of electrolytes corresponding to the decrease in volume of body water (Gamble, '29). Recent studies, however, indicate that minor degrees of dehydration are not necessarily attended by disturbance of salt balance (Wiley and Wiley, '33).

Reduction in expenditure of energy (since there was no fever) must have occurred in the operative period due to the enforced rest in bed. A reduction in caloric intake also occurred at this time due to the single day of fasting at the beginning of the period and the slight decrease in the intake of food on the remaining days. When an increment of 15 per cent was added to the basal metabolism of the patient to allow for movements in bed and to cover the specific dynamic action of the food ingested, a rough approximation of the energy expended in the operative period was obtained. The estimated production of heat, 9400 calories, was found to be nearly the same as that of the caloric intake, which for the period amounted to 9700 calories. Overfeeding, then, could hardly have been a factor in the gain in weight or retention of salt. Furthermore, it is to be recalled that the retention of sodium and potassium occurred at a time when their levels of intake had been reduced and hence the balance seems to have been opposite in direction to that which might have been expected.

Exclusion of a possible slight disturbance of thyroid function, incident to manipulation of the gland at operation, as a cause of retention of base seems justifiable. In support of this contention we cite again the quite dissimilar experiments of Greenwald and of Albright and collaborators where the only common factor which could have been held responsible for retention of base was the sudden reduction in the amount of parathormone made available to the body.

The response of our patient by retention of a large part of the salt administered as saline solution on the day of operation was qualitatively similar to that of an edematous individual who ingests a solution of sodium chloride (Schittenhelm and Schlecht, '19) or to that of a normal subject whose intake of salt has been restricted (Vallery-Radot, '18; Veil, '23; Loeb, Atchley, Benedict and Driscoll, '32; Wiley, Wiley and Waller, '33). In the present instance no rigorous restriction of salt preceded operation; the daily intake of sodium chloride averaged 91 m.eq. in preoperative control periods and this amount was increased to 152 m.eq. on the day of operation. Subsequently it was reduced to 57 m.eq. for the remainder of period 31. Unfortunately, differences in the mode of administration and in the total quantity of salt given do not permit any close analogy with previous work.

Approximately half of the potassium retained in period 31 was re-excreted in period 32, but there was no loss of sodium or chlorine. A decrease in weight accompanied the loss of potassium. Both of these effects seem to have been contingent upon a further reduction in the caloric intake, especially since the activity of the patient had been considerably increased by allowing her to get out of bed and walk about the room. With resumption of the diet of the fore periods equilibrium was again slowly attained, but only after retention of more than enough potassium to balance losses sustained in period 32.

SUMMARY

Evidence has been presented which indicates that the extirpation of a parathyroid tumor is accompanied by a temporary derangement of the metabolism of salt and water. It was found that:

1. In addition to the well-recognized alteration in the concentrations of calcium and inorganic phosphorus in the serum and their retention in the body, there was also retention of significant amounts of sodium, potassium, magnesium and chlorine.
2. The retention of the latter group of elements since accompanied by a definite increase in weight strongly suggests an increase in the volume of body water as part of the mechanism involved in readjustment.
3. The temporary nature of the disturbance of salt and water balance was attested by the fact that approximate equilibrium between intake and outgo of sodium and potassium occurred on the fifth or sixth post-operative day and hence coincided with the time at which the concentrations of calcium and inorganic phosphorus of the blood and their excretion in the urine were definitely established at normal levels.
4. While retention of the principal solutes of body fluids was found to follow removal of a parathyroid tumor, an approximately quantitative measurement of the exchanges of sodium, potassium and chlorine did not support the view that alterations in the metabolism of these elements is of primary importance in hyperparathyroidism.

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A STATISTICAL STUDY OF THE METABOLISM OF THE FASTING ALBINO RAT

JOHN C. KRANTZ, Jr., AND C. JELLEFF CARR

Department of Pharmacology, School of Medicine, University of Maryland, Baltimore

ONE FIGURE

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During the course of the investigation of certain polyatomic alcohols and their anhydrides as food stuffs for the albino rat, the authors (Carr et al., '33; Carr and Krantz, '34; Carr et al., '34) determined the respiratory quotient and heat production of nearly 100 fasting animals. These data were thought to be of sufficient interest to assemble in one communication.

Similar studies generally on a much smaller number of animals have been conducted by Benedict and MacLeod ('28) Wesson ('30) and Cori and Cori ('26).

EXPERIMENTAL

With certain minor modifications, Haldane's open circuit (1892) apparatus was employed. Male rats were used in these experiments and prior to the determination were kept at 28°C. and fed a balanced diet. They were fasted 48 hours prior to the experiment. The animal was then placed in the respiratory chamber.

Air was blown at the rate of 2 liters a minute through two scrubbing bottles of moist soda lime no. 4 mesh and one bottle of calcium chloride, and then through pumice, which had been thrust into sulphuric acid at bright red heat according to the Haldane technic. This air passed over the animal for 20 to

30 minutes. After this period the metabolism chamber was closed, the air entrapped within having been adjusted to 28°C. ± 0.5°. The metabolism chamber and animal were weighed on a balance with a load capacity of 2000 gm. and a sensitivity of 1 mg. Third decimal place weighings were employed.

The metabolism chamber was then inserted in the air line. The exit tube was connected with a series of absorption bottles containing sulphuric acid and pumice for water absorption and a soda-lime bottle and sulphuric acid pumice bottle for the absorption of carbon dioxide. Check absorption bottles for water and carbon dioxide were kept in the chain and weighed with each determination, as a check on the efficiency of the apparatus. The intake air was checked by blank runs before each series of six determinations. In this set up, the efficiency of the water absorption apparatus is worthy of special comment. After thirty runs (of 2½ hours each) the check water absorption bottle did not increase in weight significantly, i.e., ± 0.001 gm. The soda-lime required remoistening or replenishing after ten or twelve runs. Each absorption bottle for water contained about 600 gm. of the pumice-sulphuric acid mixture: those containing soda-lime had slightly less than 500 gm. of that substance in each. Five hundred cubic centimeter Woulff bottles were employed, the intake tubes to these bottles passed through the central opening in the bottle. All stoppers and rubber connections were closed with sealing wax.

No correction was made for the nitrogen content of the urine of the animals as did Cori and Cori ('26), who showed that approximately 90 per cent of the metabolism in a fasting rat is fat oxidation. The determinations were conducted between 10 A.M. and 4 P.M., according to the suggestion of Horst et al. ('34). The surface area was calculated by the formula set forth by Diack ('30), $S = 7.64 \times W^{2/3}$ for fasted rats.

DISCUSSION OF RESULTS

The data given in the graph are obtained from rats that are apparently normal. Occasionally an animal selected for a metabolism experiment was found to have an exceptionally

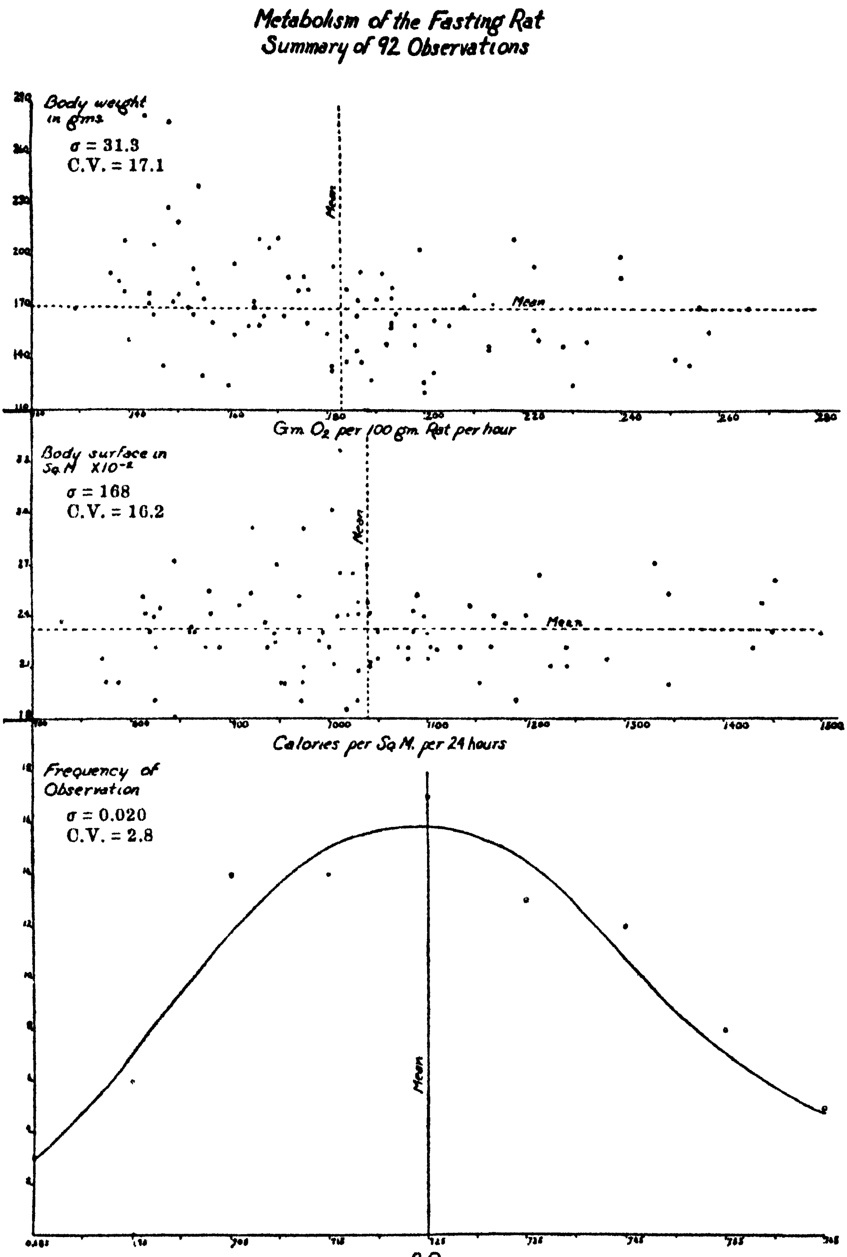
Metabolism of the Fasting Rat
Summary of 92 Observations
Body weight in gms.
σ = 31.3
C.V. = 17.1
290
260
230
200
170
140
110
Mean
Mean
120
140
160
180
200
220
240
260
280
Gm. O₂ per 100 gm. Rat per hour
Body surface in Sq. M. X10⁻²
σ = 168
C.V. = 16.2
33
30
27
24
21
18
Mean
Mean
700
800
900
1000
1100
1200
1300
1400
1800
Calories per Sq. M. per 24 hours
Frequency of Observation
σ = 0.020
C.V. = 2.8
18
16
14
12
10
8
6
4
2
Mean
0.685
.695
.705
.715
.725
.735
.745
.755
.765
R.Q.

Figure 1

high or exceptionally low respiratory quotient; such animals were discarded. Cori and Cori ('27) have pointed out the influence of season, ketosis and infection on the respiratory quotient of rats. The average values obtained then are for apparently normal rats under very carefully controlled conditions.

An examination of the frequency distribution of the respiratory quotients of the rats shows the median value practically identical with the mean of 0.725. The skewness of the curve is practically zero. The modal series falls within 0.720 and 0.729.

The mean respiratory quotient was not corrected for urinary nitrogen. Cori and Cori ('26) set forth a group of twenty-four respiratory quotients for the albino rat. Their mean value was 0.711 ± 0.005. Accepting the urinary excretion value of Cori and Cori as 3.18 mg. per 100 gm. of rat per hour, and correcting the respiratory quotient accordingly, a mean of 0.708 is obtained for the non-protein respiratory quotient.

The mean of the number of calories of heat production is 1040. This is somewhat higher than the values found by other workers. At 28° for the white rat Benedict and MacLeod ('28) found the value not far from 880 calories. These lower values are obtained when the factor 9.13 is employed in the surface area formula instead of 7.64 as recommended by Diack. Employing this older value in the calculation of heat production, the value obtained by the authors agrees well with those obtained by Benedict and MacLeod. The higher value obtained by the use of the Diack formula gives a heat production value which more closely corresponds to the value for man and other animals.

SUMMARY

The heat production and respiratory quotient of ninety-two fasting white rats have been determined under standard conditions.

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THE VITAMIN CONTENT OF SULTANINA (THOMPSON SEEDLESS) GRAPES AND RAISINS

AGNES FAY MORGAN, LOUISE KIMMEL, ANNA FIELD AND
PAUL F. NICHOLS
*Laboratory of Household Science and Fruit Products Laboratory,
University of California, Berkeley*

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In pursuance of a research begun in 1927 upon the vitamins of dried fruits, Thompson seedless grapes of the crop of 1931 and raisins prepared from them were preserved for examination as to their vitamin values.

Only a few reports of such studies upon grapes and raisins have appeared. Dutcher and Outhouse ('23) examined Thompson seedless, loose Muscat and seeded Muscat raisins with negative results as to vitamins A and C, but positive as to vitamin B, then still undifferentiated. They made no tests upon corresponding fresh grapes. Randoin ('28) reported low vitamin C content in grape juice and wines, little antineuritic B, but appreciable amounts of antiberiberi B. The nomenclature is confusing, but in the light of later work it would appear that the former is vitamin G(B_2) and the latter B(B_1). Daniel and Munsell ('32) examined Thompson seedless, Malaga grapes and two brands of commercial grape juice for vitamins A, B, C and G. They did not include raisins in their study.

As has been the custom in our researches on dried fruit the Thompson seedless grapes were picked fresh, placed in cans, evacuated twice and the vacuum released with nitrogen then kept stored at 0°C. Five lots of raisins were prepared from the similar fruit by methods outlined in table 1. The so-called soda-dipping consisted of dipping the fruit for about 30 seconds in lye held at 180°F. and then spreading on trays. Two lots, HS4 and HS6, were exposed to the fumes of burning sulfur for 5 hours before being dried.

Experimental methods. The technic employed for some years in this laboratory and previously described was used in the tests for vitamins A and C (Morgan, Field and Nichols, '31). For vitamin B(B_1) the method of Chase and Sherman ('31) was used with rats and the prophylactic method of Salmon ('27) with pigeons. Such tests as were made for vitamin G (B_2) were made by a modification of the method of Bourquin and Sherman ('31).

TABLE 1

Preparation and composition of Sultanina (Thompson seedless) grapes and raisins

LOT	LYE DIPPING	SULFURING	METHOD OF DRYING	MOISTURE	SULFUR DIOXIDE	pH	SHRINKAGE RATIO
	seconds	*hours*		*per cent*	*p.p.m.*		
HS1	..	..	Fresh grapes not dried, kept frozen	75.3	....	4.00	1:1
F.G.	..	..	Fresh grapes from market	73–76			1:1
HS2	..	..	7 days in sun, stacked after 16 days, boxed after 48 days	16.6	....	4.00	3.34:1
HS3	30	..	Same as HS2	15.8	....	4.00	3.41:1
HS4	30	5	Same as HS2	15.8	1350	3.90	3.41:1
HS5	30	..	Dehydrated at 120–160°F. for 38 hours, relative humidity 65–25 per cent, air velocity 500–700 feet per minute, boxed after 4 days	12.0	....	3.95	3.56:1
HS6	30	5	Same as HS5	11.8	1640	4.00	3.57:1

VITAMIN B ASSAY

Rat technic. The basal diet for rats consisted of purified casein 18, Osborn and Mendel salts 4, autoclaved yeast 15, butterfat 8, cod liver oil 2, corn starch 53. Animals receiving this diet and 0.05 cc. daily of a rice polish concentrate made according to the method of Evans and Lepkovsky ('31) were able to grow practically at the normal rate. The rats were placed on the basal diet at 28 days of age and after 2 or 3 weeks, when their weights plateaued, were fed weighed amounts of the fruit for 56 days.

Pigeon technic. Young but mature male pigeons were confined in small individual cages and fed by hand daily 8 gm. of baked polished rice and 8 gm. of an autoclaved mash made up of casein 19, polished rice 68, agar 2, calcium phosphate 0.5, calcium carbonate 1, sodium chloride 0.5, autoclaved yeast 6, cod liver oil 3. Enough vitamin B containing food must be given along with this ration to maintain weight and prevent disgorging, paralysis and other symptoms of polyneuritis. The pigeons were first fed the basal diet alone until they showed some of these symptoms and were then given varying doses of wheat germ in order to determine their vitamin requirement. After 2 to 4 weeks on a given dose if no untoward symptoms appeared, the dose was decreased by 10 per cent and given for another 2 to 4 weeks. The first symptom observed, usually within 3 to 7 days after the dose was lowered below the necessary minimum, was disgorging of the food. When this occurred on 2 or more successive days along with weight loss, it was assumed that the preceding dose, 10 per cent larger, had been the minimum. Restandardizing of the birds was found to be necessary every 4 or 5 months, but the same birds could be used again and again.

As has been shown by Cowgill, Deuel, Smith, Klotz and Beard ('32), a rough relationship was found to exist between the minimum pigeon dose and the rat maintenance unit. In our colony the pigeon daily dose appears to be six or seven times the daily dose required to produce 25 gm. gain in 8 weeks in young rats. The formula of Cowgill and co-workers applied to rats and pigeons of the weights used in our experiments gives a lower ratio, about 4.5. In terms of the International vitamin B_1 standard adsorbate, the pigeon dose was 50 mg., whereas the rat dose of 10 mg. produced only 34 gm. gain in 8 weeks. There is some uncertainty involved in attempting such comparisons of maintenance in adult birds with subnormal growth in young rats.

As is shown in table 2 the fresh frozen grapes were distinctly inferior in vitamin B value to similar fresh grapes purchased two or three times a week in the local market. Apparently, the removal of air from the cans containing the frozen grapes is not protective of this vitamin as it is usually of A and C. We have no explanation of this deterioration which was also observed, contrary to the usual experience, in the case of vitamin A and possibly also of vitamin C. Some

TABLE 2

Vitamin $B(B_1)$ content of Sultanina (Thompson seedless) grapes and raisins by rat assay

FRUIT	LOT NO.	AMOUNT FED DAILY	EQUIVALENT IN FRESH FRUIT	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN OR LOSS	PERIOD	RETENTION OF VITAMIN B OF FRESH GRAPES
		gm.	*gm.*		*gm.*	*gm.*	*gm.*	*days*	*per cent*
Fresh (frozen) seedless Thompson grapes	HS1	1.0	1.0	4	81	52	−29	12	37
		1.5	1.5	8	62	63	1	38	
		2.0	2.0	2	77	71	−6	49	
		3.0	3.0	3	75	84	9	56	
Fresh seedless Thompson grapes from market	F.G.	3.0	3.0	11	59	83	24	56	100
		4.0	4.0	4	58	91	33	56	
		5.0	5.0	6	66	113	47	56	
Sun-dried raisins, natural	HS2	1.5	5.0	5	57	85	28	56	60
		2.0	6.7	6	73	104	31	56	
		2.5	8.3	9	74	116	42	56	
Soda-dipped sun-dried raisins	HS3	1.0	3.4	5	71	92	21	56	94
		1.5	5.1	10	56	104	48	56	
		2.0	6.8	7	47	105	58	56	
Soda-dipped dehydrated raisins	HS5	1.5	5.3	11	63	87	24	56	56
		2.0	7.1	10	54	84	30	56	
Soda-dipped dehydrated sulfured raisins	HS6	1.5	5.3	3	61	44	−17	22	0
		2.0	7.1	10	54	43	−21	28	
		3.0	10.7	10	67	52	−15	30	
None				23	50	46	−4	25	
International standard		0.01		12	65	99	34	56	

enzyme active under the conditions of storage described may be postulated. The fresh grapes contain 1 Sherman unit in 3 gm. or 1 International unit in 4 gm.

The best retention of the original vitamin B of the grapes was shown by the soda-dipped sun-dried raisins, HS4, and about equal retention 56 and 60 per cent by the dehydrated dipped raisins, HS5, and the natural undipped sun-dried fruit, HS2. Possibly the soda dipping improved digestibility of HS3 somewhat over that of HS2.

Almost complete loss of antineuritic value was seen in HS6 the dehydrated sulfured raisins. This was confirmed by pigeon tests also upon HS4, sun-dried sulfured raisins, and later by comparison of sulfured and unsulfured peaches, figs and rice polish concentrate. Whether this destructive action concerns only $B(B_1)$ or another factor also, perhaps B4, cannot be stated at this time. Certainly, the effect of the sulfur dioxide is more disastrous in the rat than in the pigeon tests.

An experiment designed to test the effect of SO_2 on the vitamin more definitely was recently carried out.[1] The rice polish concentrate already mentioned was exposed to the action of washed sulfur dioxide gas by bubbling the gas from a commercial cylinder through the diluted extract for 1 hour at room temperature. The retained SO_2 was largely removed by evacuation from the concentrate, but a measurable amount remained as may be seen in table 3. Attempts were made to recover the vitamin B activity of the SO_2-treated extract by bubbling CO_2 through it for 3 hours and by treatment with barium chloride with subsequent removal of the barium with sodium sulfate.

As may be seen in table 3, none of the three types of treated extract appeared to retain antineuritic activity in any degree.

A few tests upon pigeons with the SO_2-treated extract have likewise proved negative, although by sufficiently increasing the dose it has been possible to defer or entirely prevent polyneuritis. Apparently, the vitamin B_1 destruction is not complete, although another factor, possibly B_4, needed by rats and not by pigeons, may be entirely destroyed by the treatment.

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[1] The help of Elizabeth Ludlow Fawkes in this part of the experiment is gratefully acknowledged.

It is interesting to speculate upon the mechanism of this action of sulfur dioxide. The vitamin may be reduced, hydrolyzed or a sulfite product formed. The theory that hydrolysis by sulfurous acid may occur seems improbable in view of the well-known resistance of the vitamin to the action of even stronger acids. Since basic properties are usually ascribed to vitamin B, an inactive sulfite may be postulated, but the failure of reactivation by barium treatment or carbon dioxide speaks against this.

TABLE 3

Vitamin B value of rice polish extract as affected by sulfur dioxide

DAILY AMOUNT FED	TREATMENT	pH	SO_2	NUMBER OF RATS	WEIGHTS			PERIOD OF TEST
					Initial	Final	Loss or gain	
gm.			*p.p.m.*		*gm.*	*gm.*	*gm.*	*days*
0.025	Untreated extract	5.4		11	59	90	31	56
0.050				11	71	157	86	56
0.100				5	70	225	155	56
0.025 to 0.200	SO_2 treated	5.8	1570	12	78	56	—22	20
0.1 and 0.2	SO_2 treated and Ba ppt or CO_2 saturated	6.2	740 1380	10	76	49	—27	24
	No vitamin B			28	53	46	—7	22
0.010	International standard			12	65	99	34	56

There remains the reducing action of the SO_2. Kramár ('24) reported loss of antineuritic activity as resulting from drastic reduction of alcoholic extracts of yeast by aluminum amalgam in alkaline medium, but failure of such loss of activity when zinc-hydrochloric acid in acid medium was used as the reducing agent. Control experiments seemed to show that the alkali alone was not responsible for the destruction in the former case. Another significant finding is that of

Levene and Van der Hoeven ('25), who increased the potency of the yeast extract product obtained by preliminary precipitation with basic lead acetate by removal of the lead with sulfuric acid instead of hydrogen sulfide. Failure to remove the active substance from the precipitate when the latter reagent was used might be due to its destruction through reduction.

No other attempts at reduction of vitamin B have been found reported in the literature. The present study may be interpreted as evidence in favor of the easy destruction of the vitamin by reducing action. This is in interesting contrast with the easy destruction by oxidation of certain other vitamins, namely, A and C, and probably also, but in less degree, D and E.

In table 4 the detail of some of the observations upon pigeons is given and in table 5 a summary of the findings by this method. The results agree remarkably well with those obtained with rats, with the exception that the frozen fruit appeared undeteriorated. This may be due to the fact that these were the first tests made and that the grapes may have been as yet unaffected.

In any case the unsulfured raisins contained 1 Sherman unit in 1.0 to 1.5 gm. of fruit or 1 International unit in 1.5 to 2.0 gm. and a pigeon maintenance unit in 7 gm. The distinctly lower values seen by Daniel and Munsell ('32) in their Thompson seedless grapes resemble our figures for the frozen stored grapes, which doubtless their samples, purchased in Washington, D. C., after storage in transit from California, resembled more than they did the freshly gathered grapes from the Berkeley market.

VITAMIN A ASSAY

Again, when the vitamin value of the frozen grapes was tested it was found that a very considerable decrease, about 50 per cent, had occurred in frozen storage. This was somewhat surprising because the fruit was kept in air-evacuated cans. The change which occurred was apparently anaerobic in

TABLE 4

Pigeon tests for vitamin B assay of raisins and grapes

NUMBER OF BIRD	PERIOD	WEIGHT OF BIRD	MATERIAL FED	DAILY DOSE	REMARKS
	days	*gm.*		*gm.*	
15	29	404–453	HS3	7.0	Normal
	16	451–445	HS3	6.3	
	12	442–438	HS3	6.7	Disgorged 5 times
	9	427–445	HS3	7.0	Normal
	21	450–393	HS3	6.7	Disgorged 5 times, polyneuritic
	31	391–416	HS2	8.0	Normal
	5	416–418	HS2	7.0	Disgorged 3 times
	19	403–409	HS6	8.0 and 0.3 gm. wheat germ	Disgorged 11 times
	24	409–431	HS6	8.0 and 0.4 gm. wheat germ	Disgorged 4 times, polyneuritic on 24th day. Given 3 gm. yeast
	37	421–446	HS6	8.0 and 0.5 gm. wheat germ	Normal
	27	446–441	HS6	8.0 and 0.4 gm. wheat germ	Disgorged on 19th and 27th days
3	60	435–369	Fresh frozen seedless Thompson grapes (HS1)	4–11	Disgorged, lost weight. Insufficient protection
	30	356–382	Fresh frozen seedless Thompson grapes (HS1)	16.0	Normal
	25	382–404	Fresh frozen seedless Thompson grapes (HS1)	14.4	Normal
	30	377–395	Fresh market grapes	15.8	Disgorged 5 times
	14	377–382	Fresh market grapes	17.0	Normal
2	33	452–404	HS1	10–17	Disgorged constantly
	36 3	394–400	HS1 HS1	18.0 16.2	Nearly normal Disgorged
	8	396–392	HS6	8 and 0.4 gm. wheat germ	Constant disgorging
	30	392–409	HS6	8 and 0.5 gm. wheat germ	Normal

TABLE 4—*Continued*

NUMBER OF BIRD	PERIOD	WEIGHT OF BIRD	MATERIAL FED	DAILY DOSE	REMARKS
	days	*gm.*		*gm.*	
	10	412–398	HS6	8 and 0.4 gm. wheat germ	Disgorged
14	19	401–431	HS2	7.0	Disgorged on 19th day
	43	424–403	HS2	8.0	Nearly normal
	7	401–376	HS2	7.0	Disgorged 3 times
	30	376–394	HS3	8.0	Normal
	30	392–398	HS3	7.0	Normal
	27	398–410	HS3	6.0	Disgorged after 27 days
16	30	406–385	HS4	7 to 13	Frequent disgorging
	8	343–356	HS4	7 and 0.45 gm. wheat germ	Normal
	30	351–394	HS6	8 and 0.45 gm. wheat germ	Normal
	30	394–393	HS6	6.4 and 0.45 gm. wheat germ	Normal
	8	388–387	HS6	6.4 and 0.3 gm. wheat germ	Constant disgorging
	36	371–404	HS5	8.0	Normal
	30	406–412	HS5	7.0	Normal
	7	413–408	HS5	6.0	Disgorged
10	30	386–397	HS5	8.0	Normal
	30	393–412	HS5	7.0	Normal
	10	416–415	HS5	6.0	Disgorged 3 times
	35	425–437	Wheat germ	0.70	Normal
	29	435–442	Wheat germ	0.63	Disgorged on 29th day

TABLE 5

Retention of vitamin B by Thompson seedless raisins as shown by pigeon assay

NUMBER OF BIRD	DAILY DOSE REQUIRED FOR MAINTENANCE				RETENTION OF VITAMIN B OF FRESH GRAPES
	Wheat germ	Fruit			
		Type	Amount	Equivalent in fresh fruit	
	gm.		*gm.*	*gm.*	*per cent*
10	0.7	HS5	7.0	25	64
16	0.9	HS5	7.0	25	64
15	0.8	HS3	7.0	24	66
14	0.9	HS3	7.0	24	66
14	0.9	HS2	8.0	27	59
15	0.8	HS2	7.0	23	69
15	0.8	HS6	8.0 and 0.5 gm. wheat germ	85	18
2	0.7	HS6	8.0 and 0.5 gm. wheat germ	85	18
16	0.9	HS6	6.4 and 0.45 gm. wheat germ	65	24
16	0.9	HS4	7.0 and 0.45 gm. wheat germ	68	23
2	0.7	HS1	18.0	18	100
3	0.7	HS1	14.4	14	
3	0.7	Fresh grapes	17.0	17	

character as it must also have been in the case of vitamin B. That it was progressive is evinced by the better response on 2 gm. doses of the frozen fruit carried out soon after the samples were procured than on 3 gm. doses made several months later. The fresh grapes appeared to contain 1 International unit (allowing 6 to 8 gm. gain per week for 8 weeks) in 2 gm. (table 6).

TABLE 6

Vitamin A content of Sultanina (Thompson seedless) grapes and raisins

FRUIT	LOT NO.	AMOUNT FED DAILY	EQUIVALENT IN FRESH FRUIT	NUMBER OF RATS	AVERAGE WEIGHTS Initial	AVERAGE WEIGHTS Final	AVERAGE GAIN PER WEEK	EXPERIMENTAL PERIOD	WEIGHT YIELDING 1 UNIT (INTERNATIONAL) IN FRESH FRUIT EQUIVALENT	VITAMIN A RETENTION
		gm.	*gm.*		*gm.*	*gm.*	*gm.*	*days*	*gm.*	*per cent*
Fresh grapes (frozen)	HS1	1.0	1.0	9	107	112	0.9	42		
		2.0	2.0	7	109	142	4.1	56	4.0	50
		3.0	3.0	3	120	148	3.5	54		
Fresh grapes	F.G.	3.0	3.0	4	103	187	10.5	56	2.0	100
		5.0	5.0	4	114	214	12.5	56		
Sun-dried raisins, natural	HS2	1.0	3.3	4	98	92		15		0
		2.0	6.7	6	107	92		18		
		3.0	10.0	13	98	91		24		
Soda-dipped sun-dried raisins	HS3	2.0	6.8	5	106	132	3.2	56		
		3.0	10.2	7	101	139	4.8	56	13.6	15
Soda-dipped sulfured sun-dried raisins	HS4	1.0	3.4	6	105	95		35		
		1.5	5.1	2	104	113	2.2	28		
		2.0	6.8	5	109	147	4.8	56	10.2	20
Soda-dipped dehydrated raisins	HS5	0.5	1.8	7	118	170	6.5	56	2.0	100
		1.0	3.5	9	112	196	10.5	56		
		1.5	5.3	5	111	201	11.2	56		
		2.0	7.1	7	95	199	13.0	56		
Soda-dipped sulfured dehydrated raisins	HS6	0.5	1.8	7	112	172	8.2	51	2.0	100
		1.0	3.5	12	111	207	12.0	56		
		1.5	5.3	6	97	205	13.5	56		
		2.0	7.1	8	110	206	12.0	56		
		mg.							*mg.*	
Carotene, International standard		0.001		12	104	156	6.5	56	0.001	

None of the sun-dried raisins, HS2, HS3 or HS4, showed more than a modicum of vitamin A. However, the two dehydrated specimens, HS5 and HS6, the latter sulfured, appeared to retain the full vitamin A content of the fresh fruit. This was the most decisive contrast so far seen between dehydrated and sun-dried fruit in this respect. The natural sun-dried raisins, HS2, were wholly lacking and the two soda-dipped products, HS3 and HS4, retained only 15 and 20 per cent of the vitamin of the fresh fruit. Such sensitivity to the exposure and heat of sun-drying was to be expected perhaps in a fruit so subject to enzyme changes.

The soda dipping appeared favorable, HS3 compared with HS2, perhaps because of better absorbability. The sulfuring appeared slightly favorable also, HS3 compared with HS4.

Again, the findings of Daniel and Munsell ('32) and Dutcher and Outhouse ('32) were less favorable than ours perhaps because of the losses in storage of the fresh grapes in the former case and the failure to test dehydrated raisins in the latter.

VITAMIN C ASSAY

The frozen grapes showed progressive loss of antiscorbutic property for, as shown in table 7, 20 gm. doses were more protective at first than 25 gm. doses later. Even fresh market grapes, however, did not afford full protection to the guinea pigs in 30 gm. daily doses. None of the raisin products were protective. It must be concluded that this variety of grape is not well endowed with vitamin C.

VITAMIN G CONTENT

An attempt was made to assess the vitamin G(B_2) content of these products, but without satisfactory conclusions. The daily doses fed were probably in most cases too small to give significant differences in the resulting growth. Fresh grapes in 2 gm. daily doses made an appreciable increase in the growth of rats fed the vitamin G-free basal diet as did 1 gm. doses of the raisins. The fruit appears rather poorly endowed in this vitamin—a fact observed in the fresh grapes by Daniel and Munsell ('32). There is apparently about 1 unit (Bourquin-Sherman) of vitamin G in 2 gm. of the raisins, whether sulfured or unsulfured. A more decisive experiment on this point is yet to be undertaken.

TABLE 7

Vitamin C content of Sultanina (Thompson seedless) grapes and raisins

FRUIT	AMOUNT FED DAILY		NUMBER OF GUINEA PIGS	BODY WEIGHTS			PERIOD	SCURVY SCORE[1]
	Grams	Equivalent in fresh fruit		Initial	Final	Gain or loss		
		gm.		*gm.*	*gm.*	*gm.*	*days*	
HS1 (frozen grapes)	10, 15	10, 15	15	336	270	—66	47	10
	20	20	2	284	337	+53	60	1
	25	25	4	322	238	—84	41	9
Fresh grapes	30	30	3	318	372	+54	52	3
HS2 (natural raisins)	6, 8	20, 27	4	342	282	—60	53	5
HS3 (soda-dipped natural raisins)	4, 6	14, 20	6	329	244	—85	34	15
HS4 (sulfured natural raisins)	4–10	14–34	15	324	214	—110	39	12
HS5 (dehydrated raisins)	10	36	4	326	254	—72	38	14
HS6 (dehydrated sulfured raisins)	4–10	14–36	18	328	225	—103	52	11
	15	53	2	335	192	—143	60	9

[1] 0, no scurvy to 24, most severe scurvy.

DISCUSSION

Some interest of a practical as well as theoretical character attaches to the divergent effect of the sulfuring process upon the various vitamins. That such differences would be found in the effect of sulfur dioxide is to be expected, since each of the vitamins so far chemically identified is distinct in nature from all the others. Thus vitamins A and C are known to be readily oxidized labile molecules which, as has been found, might well be protected from destruction by a reducing substance such as sulfur dioxide. On the other hand, vitamin B which is probably a base, not readily oxidized, but heat-labile, might be injured by the same substance. There is some previous evidence that this vitamin is readily reduced (Kramár, '24). Guha ('31) has stated that vitamin G(B_2) is not affected by sulfur dioxide and so far as our observations have gone this is true of the vitamin G of dried fruit.

There is no single rule which can be set forth as embodying the best practice in drying methods for all fruits or for all vitamins. Probably each fruit should be preserved in that fashion which conserves its best vitamin endowment. Thus peaches and apricots, which are excellent sources of A and C and probably poor sources of B, should be sulfured, but raisins and prunes which are good sources of B and poor sources, at least of C, should not be sulfured. Dehydration rather than sun-drying is advisable in addition if the vitamin A is to be adequately conserved.

SUMMARY

1. Seedless Thompson grapes when preserved by freezing storage, even after air evacuation, rapidly lose both vitamin A and B activities.

2. These grapes are rather well endowed with vitamin B when fresh and this endowment is largely retained in the natural sun-dried and soda-dipped raisins which contain 1 International (or about 1.4 Chase and Sherman) vitamin B unit in 1.5 to 2.0 gm. However, treatment of the fruit with sulfur dioxide previous to drying destroys much of the vitamin. Such destruction is more striking if shown by rat growth instead of by pigeon maintenance.

3. The fresh grapes are richer in vitamin A than has been hitherto supposed possible, because really fresh fruit has not previously been examined. The dehydrated raisins, both sulfured and unsulfured, retained this factor in full, 1 International unit in 0.5 gm. All the sun-dried raisins had lost most of the vitamin A activity.

4. The frozen grapes appeared to lose antiscorbutic activity in storage and even the fresh grapes were only poorly endowed

with vitamin C. Very little protective value could be ascribed to any of the raisins.

5. Vitamin G appears to be poorly represented in grapes and raisins, but conclusive experiments are not yet available.

6. Unsulfured soda-dipped dehydrated raisins made from Thompson seedless grapes retain practically all of the considerable vitamin A and B activity of the fresh grapes.

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THE VITAMIN CONTENT OF FIGS

AGNES FAY MORGAN, ANNA FIELD, LOUISE KIMMEL AND PAUL F. NICHOLS

Laboratory of Household Science and Fruit Products Laboratory, University of California, Berkeley

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Figs of four varieties and three different crop years were used in the series of experiments here reported. The usual method (Morgan and Field, '29) developed for the study of the vitamin content of other dried fruits was followed in all cases. Fresh fruit was selected under known conditions, kept in frozen storage, and samples of similar fruit dried under varying conditions were examined as to vitamin activity along with the fresh. An attempt was thus made to discover the probable original vitamin content of the fruit as well as the effect upon this of the preserving methods used. No record of other vitamin studies upon figs has been found.

Kadota and Calimyrna figs were examined in 1929, Missions in 1930 and Kadotas and Adriatics in 1932. As shown in table 1, seventeen samples were prepared, six fresh and eleven dried, eight of the latter being sulfured. Only one sample, H12, was dehydrated, all others being sun-dried, as is the prevailing commercial fashion. Another factor of difference lay in the stage at which the fruit was picked. The fresh samples H9, H8, S5, F1 and F4 were picked from the trees at the stage suitable for consumption, and F7 was allowed to shrink to some extent before being picked. Certain of the dried products, H10, S6, S7, F8 and F9, were gathered after the natural drop from the trees, but the others, H11, H12, F2, F3, F5 and F6, were picked fresh and juicy and then subjected to the drying process. Only perfect fruits were used.

TABLE 1

Preparation and analysis of fig samples

VARIETY	YEAR	LOT NO.	PREPARATION	MOISTURE	SO_2	pH	DRYING RATIO
				per cent	*p.p.m.*		
Kadota	1929	H9	Fresh, ground, kept frozen in storage after evacuation and release of vacuum with nitrogen	68.2	....	4.6	1:1
Calimyrna	1929	H8	Fresh, prepared as was H9	65.2	....	4.3	1:1
		H10	Sun-dried 10 days, natural drop	12.6	....	4.6	2.5:1
		H11	Sulfured 3 hours, sun-dried 10 days	14.1	1035	4.1	2.4:1
		H12	Sulfured 3 hours, dehydrated for 24 hours at temperatures from 120° to 150°F., relative humidity from 60 to 20 per cent, air velocity 900 feet per minute	12.9	1438	3.9	2.5:1
Mission	1930	S5	Fresh, prepared as was H9	67.5	....	4.8	1:1
		S6	Sun-dried, natural drop	19.4	....	4.6	2.5:1
		S7	Picked fresh, sulfured 1 hour, sun-dried	18.3	195	4.3	2.5:1
Kadota	1932	F1	Fresh, prepared as was H9	76.2	....	4.5	1:1
		F2	Picked fresh, sulfured 7.5 hours, sun-dried	19.7	925	4.2	3.4:1
		F3	Picked fresh, dipped 90 seconds in 2 per cent boiling lye, washed, sulfured 7.5 hours, sun-dried	16.7	1157	4.2	3.5:1
Adriatic	1932	F4	Fresh, prepared as was H9	75.9	....	4.2	1:1
		F5	Picked fresh, sulfured 5 hours, sun-dried	24.7	275	4.1	3.1:1
		F6	Picked fresh, dipped 120 seconds in 2 per cent boiling lye, washed, sulfured 5 hours, sun-dried	26.3	550	4.2	3.0:1
		F7	Picked shrunken, prepared as was H9	62.9	....	4.1	1.5:1
		F8	Natural drop, sulfured 5 hours, sun-dried	14.1	256	4.3	3.5:1
		F9	Natural drop, sun-dried	15.9	....	4.3	3.5:1

A further variable lay in the dipping process, similar to that used for prunes and some raisins, to which samples F3 and F6 were subjected. This is not used in commercial production, but was tried in order to determine its effect upon vitamin retention.

Only vitamins A and C were studied in the 1929 and 1930 samples, but vitamins B and G were also included in the 1932 experiment. The biological methods used were the same as have been previously described (Morgan and Field, '30; Morgan et al., '31; Morgan et al., '35).

VITAMIN C RESULTS

None of the figs were found to be rich in antiscorbutic property, although the fresh Kadotas and Calimyrnas of 1929 were protective when daily doses of 20 to 30 gm. were given (table 2). The Kadotas of 1932 were not examined as promptly after storage as were those of 1929 and were found less active. This difference may be due to actual crop variation or to the longer frozen storage—a factor which was found (Morgan et al., '35) in grapes to exert an injurious effect. None of the other fresh fruit, Missions or Adriatics, proved protective in the amounts which could be fed. None of the dried products, sulfured or unsulfured, dipped, picked fresh or shrunken, retained measurable amounts of vitamin C.

VITAMIN A RESULTS

As is shown in table 3, the Mission figs have considerably better vitamin A endowment than have any of the lighter colored varieties. This is not surprising, since the color of the Mission fruit hints at a probably larger content of carotene. The sulfuring process was seen to increase the retention of this vitamin in the dried Mission figs, S7, sulfured, retaining about 33 per cent as compared with 20 per cent by S6, unsulfured. The same contrast may be seen in H11 and H10, dried Calimyrnas, but not in F8 and F9, dried Adriatics.

As has been demonstrated in raisins (Morgan et al., '35) and in other fruits, the dehydration process is superior to sun-drying for preservation of vitamin A, the single dehydrated fig product, H12, Calimyrnas, being more than twice as rich as any of the sun-dried fruit, even though fresh Calimyrnas were not particularly well endowed.

The lye dipping which preceded sulfuring in F6 added no advantage as it was found to do in the case of prunes.

VITAMIN B(B_1) RESULTS

Both rat growth and pigeon maintenance methods were used in the vitamin B tests. These methods have been de-

TABLE 2

Vitamin C content of fresh and dried figs

VARIETY	LOT NO.	NUMBER OF GUINEA PIGS USED	DAILY AMOUNTS FED		AVERAGE GAIN PER DAY	LENGTH OF PERIOD	SCURVY SCORE[1]
			Grams	Equivalent in fresh			
				gm.	*gm.*	*days*	
Fresh Kadota	H9	7	10	10	—2.0	28	9
		5	20	20	0.0	43	3
		1	25	25	2.5	60	0
	F1	6	25	25	—3.1	29	14
		2	30	30	—2.3	50	12
		6	35	35	—1.8	50	12
Dried Kadotas	F2, F3	4	12	41	—4.7	33	14
Fresh Calimyrna	H8	7	7 to 15	7 to 15	—2.7	39	7
		4	20 to 30	20 to 30	3.3	60	0
Dried Calimyrnas	H10, H11, H12	37	3 to 15	7 to 37	—3.6	29	15
Fresh Mission	S5	17	5 to 20	5 to 20	—3.7	33	19
Dried Missions	S6, S7	10	4 to 10	10 to 25	—3.3	33	15
Fresh Adriatic	F4	11	25 to 30	25 to 30	—1.7	44	11
		6	35	35	—1.1	58	10
Fresh (shrunken) Adriatic	F7	16	25 to 35	37 to 42	—2.7	39	11
Dried Adriatics	F5, F6, F8, F9	8	12	36 to 42	—3.8	33	13

[1] On a scale of increasing severity of symptoms, 0 to 24.

TABLE 8

Vitamin A content of fresh and dried figs

VARIETY	LOT NO.	NUMBER OF RATS USED	DAILY AMOUNT FED		BODY WEIGHTS		PERIOD	WEIGHT YIELDING 1 INTERNATIONAL UNIT IN FRESH EQUIVALENT
			Grams	Equivalent in fresh	Initial (depleted)	Gain		
					gm.	gm.	days	gm.
Fresh Kadota	H9	15	0.2 to 1.5	0.2 to 1.5	66	14	28	
	F1	5	2.0	2.0	94	51	53	2.0
		5	4.0	4.0	101	77	56	
Sun-dried, sulfured Kadota	F2	5	1.0	3.4	114	34	33	
		4	2.0	6.7	121	59	56	6.7
Fresh Calimyrna	H8	13	0.15 to 0.75	0.15 to 0.75	64	6	29	
Sun-dried Calimyrna unsulfured	H10	11	0.4 to 0.9	1.0 to 2.2	72	13	33	
		4	1.0	2.5	91	14	50	
Sun-dried Calimyrna sulfured	H11	10	0.4 to 0.9	1.0 to 2.2	81	16	35	
		3	1.0	2.5	86	36	56	3.4
Dehydrated Calimyrna sulfured	H12	7	0.45	1.1	60	38	51	
		1	0.9	2.2	65	71	56	1.6
Fresh Mission	S5	6	1.0	1.0	98	46	56	1.0
		7	1.5	1.5	76	70	56	
Sun-dried unsulfured Mission	S6	9	0.9 to 1.0	2.2 to 2.5	118	5	45	
		4	1.5	3.7	115	24	56	
		6	2.0	5.0	108	42	56	5.0
Sun-dried sulfured Mission	S7	11	0.9 to 1.0	2.2 to 2.5	117	4	50	
		8	1.5	3.2	83	50	56	3.2
		5	2.0	5.0	99	61	56	
Sulfured sun-dried Adriatic	F5	3	1.0	3.1	95	0	30	
		3	2.0	6.2	93	38	50	
Dipped sulfured sun-dried Adriatic	F6	5	1.0	3.0	96	34	51	
		4	2.0	6.1	120	5	39	
Sulfured sun-dried Adriatic	F8	5	1.0	3.5	87	29	36	
		4	2.0	7.0	105	33	41	
Unsulfured sundried Adriatic	F9	4	1.0	3.5	87	21	35	
		5	2.0	7.0	86	59	56	7.0

scribed in a previous report (Morgan et al., '35). Two sources of vitamin $G(B_2)$ were tried for the rat experiments, an autoclaved liver extract prepared under the direction of Dr. S. Lepkovsky, and the usual autoclaved yeast. The liver extract was fed separately and the yeast was incorporated in the basal diet. In general, the growth obtained with the various fig samples was much the same whichever of these vitamin G sources was used. The liver extract was used in the belief that the laxative effect of the figs and yeast together might decrease the availability of the water-soluble vitamins present. However, the liver extract was found also to produce a laxative effect upon the intestines.

The injurious effect of sulfur dioxide upon vitamin B previously recorded in the case of raisins (Morgan et al., '35) was observable in these fig tests, although in less obvious degree. As shown in table 4, containing the rat growth data, the one unsulfured dried product, F9, retained nearly twice as much of the vitamin B of the fresh fruit as did the sulfured products.

The contrast is not so marked in the pigeon maintenance results seen in table 5. It will be noted in the latter that small amounts of wheat germ were fed to supplement the fig doses in order to decrease the bulk of fruit administered. Each pigeon was standardized as to weight of wheat germ needed for maintenance of weight and prevention of disgorging or other symptoms of vitamin B deficiency. The wheat germ equivalent of the various fig preparattions could thus be rather definitely determined. In table 6 examples of the records of a few of the birds are given.

Three birds were used to determine the value of the International standard, an acid adsorbate dispensed by the Committee on Vitamin Standards of the League of Nations ('31). With the technic which has been applied in this laboratory to numerous products, based on Salmon's ('27) method, 0.05 gm. of the standard was found to be equivalent of 0.7 gm. wheat germ[1] and 0.07 gm. to be the equivalent of 1.0 gm. wheat germ.

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[1] This wheat germ is a sterilized flaked product supplied by the Sperry Flour Company, of San Francisco, and found to be of constant vitamin B content when examined by both rat and pigeon tests.

TABLE 4

Content of vitamin $B(B_1)$ of fresh and dried figs

VARIETY	LOT NO.	SO_2	DAILY DOSE		NUMBER OF RATS	INITIAL (DEPLETED) WEIGHTS	TOTAL GAIN OR LOSS	PERIOD	SOURCE OF VITAMIN G	WEIGHT PROVIDING		RETENTION OF VITAMIN B OF FRESH FRUIT
			Grams	Equivalent in fresh weight						1 International unit	Sherman unit	
										Equivalent in fresh weight		
		p.p.m.		*gm.*		*gm.*	*gm.*	*days*		*gm.*	*gm.*	*per cent*
Kadota, fresh	F1	....	4.0	4.0	6	65	11	56	Autoclaved yeast	3.8	2.8	100
			6.0	6.0	3	60	53	56				
Kadota, dried	F2	925	2.0	6.7	6	69	—10	38	Autoclaved yeast			
			2.0	6.7	6	82	—22	42	Liver extract			
			3.0	10.1	5	73	—23	20	Liver extract			
	F3	1157	2.0	7.0	6	66	— 7	41	Autoclaved yeast			
			3.0	10.5	5	87	—20	28	Liver extract			
Adriatic, fresh	F4	....	2.0	2.0	3	55	4	56	Autoclaved yeast	4.0	3.0	100
			4.0	4.0	2	80	33	56	Autoclaved yeast			
Adriatic, fresh but shrunken	F7	....	2.0	3.0	7	64	14	56	Autoclaved yeast	7.3	5.3	56
Adriatic, dried	F5	275	1.0	3.1	6	66	—12	21	Autoclaved yeast	10.9	8.0	37
			3.0	9.3	6	64	29	56	Autoclaved yeast			
			1.0	3.1	6	89	—22	38	Liver extract			
			3.0	9.3	6	87	0	56	Liver extract			
Adriatic, dried	F6	550	3.0	9.2	5	86	—20	28	Liver extract			
			2.0	6.2	6	65	7	44	Autoclaved yeast			
Adriatic, dried natural drop	F8	256	1.0	3.5	6	75	—22	24	Autoclaved yeast			
			1.0	3.5	6	88	—29	43	Liver extract			
			3.0	10.6	2	68	18	56	Liver extract	16.0	12.0	25
Adriatic, dried natural drop	F9	....	1.0	3.5	6	88	—18	52	Liver extract			
			3.0	10.5	5	78	54	56	Liver extract			
			1.0	3.5	6	69	— 4	51	Autoclaved yeast	6.5	4.8	61
International standard			0.010		12	65	34	56	Autoclaved yeast	0.010	0.007	
			0.010		4	64	24	56	Liver extract	0.010	0.010	
Rice polish concentrate			0.025		14	57	43	56	Autoclaved yeast	0.020	0.014	
			0.050		15	58	102	56	Autoclaved yeast			
			0.025		5	61	34	56	Liver extract	0.025	0.019	
			0.050		4	85	99	56	Liver extract			
Nothing					23	60	— 4	25	Autoclaved yeast			
					10	85	—36	25	Liver extract			

The rice polish concentrate, prepared according to the method of Evans and Lepkovsky ('31), which is used in this laboratory as concentrated source of vitamin B practically free from vitamin G, was similarly compared on two of the same pigeons

TABLE 5

Vitamin $B(B_1)$ content of figs as determined by pigeon maintenance test

PRODUCT	NUMBER OF BIRD	DAILY AMOUNT REQUIRED FOR MAINTENANCE					AMOUNT EQUIVALENT (FRESH) TO 0.7 GM. WHEAT GERM[1]	RETENTION OF VITAMIN B OF FRESH FRUIT
		Wheat germ alone	Weight of fruit	Equivalent in fresh weight	Additional wheat germ	Total equivalent in fresh weight		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Fresh Kadota, F1	31	1.1	28	28	....	28	18	
	15	0.8	18	18	....	18	16	100
Dried Kadota, F2	27	1.0	8	27	0.55	60	42	
	33	1.0	8	27	0.55	60	42	40
Dried Kadota, F3	28	1.0	8	28	0.55	62	43	
	31	1.1	8	28	0.55	56	36	43
Fresh Adriatic, F4	26	1.0	15	15	....	15	10	
	21	0.7	15	15	....	15	15	
	29	1.2	18	18	....	18	10	
	19	0.7	15	15	....	15	15	100
Fresh shrunken Adriatic, F7	19	0.7	20	30	....	30	30	
	22	0.7	18	27	....	27	27	44
Dried Adriatic, F5	10	0.7	8	25	0.20	35	35	
	15	0.8	8	25	0.20	33	29	
	21	0.7	8	25	0.20	35	35	38
Dried Adriatic, F6	28	1.0	8	24	0.50	48	38	
	33	1.0	8	24	0.50	48	38	33
Dried Adriatic, F8	15	0.8	8	28	0.30	45	42	
	21	0.7	8	28	0.20	39	39	
	33	1.0	8	28	0.30	40	28	35
Dried Adriatic, F9	25	1.1	8	28	0.40	44	28	
	32	0.7	8	28	0.10	32	32	
	29	1.2	8	28	0.50	48	27	
	31	1.1	8	28	0.40	44	28	43
International standard	26	1.0	0.07				0.05	
	10	0.7	0.05				0.05	
	2	0.7	0.05				0.05	
Rice polish concentrate	2	0.7	0.08				0.08	
	10	0.7	0.08				0.08	

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[1] Equivalent to 1 International vitamin B (pigeon) unit.

TABLE 6

Examples of pigeon maintenance test for vitamin $B(B_1)$ in figs

NUMBER OF BIRD	PRODUCT FED	DAILY AMOUNT	DATES 1932–1933	WEIGHT	CONDITION
		gm.		*gm.*	
19	Wheat germ	0.7	Oct. 1–Oct. 25	492–485	Normal
		0.6	Oct. 26–Oct. 27	485–482	Normal
		0.6	Oct. 28	471	Disgorged
	Fresh fig, F4	15.0	Oct. 30–Nov. 27	478–468	Normal
		12.0	Nov. 28–Dec. 2	465	Normal
		12.0	Dec. 3		Polyneuritic
	Shrunken fig, F7	20.0	Dec. 6–Jan. 18	430–470	Normal
		18.0	Jan. 19–Jan. 24	470	
		18.0	Jan. 25	472	Disgorged
22	Wheat germ	0.7	Sept. 29–Oct. 25	430	Normal
		0.6	Oct. 26–Oct. 29	433	Normal
		0.6	Oct. 30	460[1]	Polyneuritic
	Shrunken fig, F7	15.0	Nov. 3–Nov. 16	425	Occasional disgorging
		20.0	Nov. 17–Dec. 15	420–411	Normal
		15.0	Dec. 16–Jan. 1	435–404	Disgorging
21	Wheat germ	0.7	Sept 27–Oct. 25	395–400	Normal
		0.6	Oct. 26–Nov. 10	404–476	Normal
		0.6	Nov. 11	480	Polyneuritic
	Fresh fig, F4	15.0	Nov. 19–Dec. 17	386–410	Normal
		12.0	Dec. 18–Dec. 20	412–427	Polyneuritic
15	Dried fig, F8	8 + 0.4 w.g.[2]	Feb. 22–Mar. 7	414–435	Normal
		8 + 0.3 w.g.	Mar. 8–Mar. 20	435–437	Normal
		8 + 0.2 w.g.	Mar. 21–Mar. 27	438	Disgorging
	Dried fig, F5	8 + 0.5 w.g.	Mar. 28–Apr. 9	430	Normal
		8 + 0.4 w.g.	Apr. 10–Apr. 22	440–415	Normal
		8 + 0.3 w.g.	Apr. 23–May 5	426	Normal
		8 + 0.2 w.g.	May 6–May 18	430	Normal
		8 + 0.1 w.g.	May 19–June 1	420	Disgorged 4 times
	Wheat germ	0.9	June 6–June 19	410–460	
		0.8	June 20–July 2	450	
		0.7	July 3–July 6	450	Disgorging
	Fresh fig, F1	30.0	Aug. 2–Aug. 14	424–426	Normal
		27.0	Aug. 15–Aug. 28	426–406	Normal
		20.0	Aug. 29–Sept. 10	406–400	Normal
		15.0	Sept. 11–Sept. 17	414	Disgorging
		18.0	Sept. 20–Oct. 2	404–410	Normal

[1] Occasionally weight increases resulted when the crop muscles were affected so that disgorging could not occur.

[2] w.g. = wheat germ.

and 0.08 gm. found to be equivalent to one International unit. Similar comparisons of the rice polish concentrate and International adsorbate units by the rat growth method yielded somewhat similar proportions, 2.0 or 2.1 to 1 by rat growth, and 1.6 to 1 by pigeon maintenance.

The amount of the various fig preparations required to protect the birds in the same degree as did the International adsorbate could thus be calculated even though the full amount required could not in all cases be fed. Apparently, the vitamin of the fresh Kadota figs was less available to the pigeons than to the rats, for the average pigeon unit amount was larger, 17 gm., than in the case of the fresh Adriatics, 12.5 gm. For the rats the unit amounts of these two kinds of figs were nearly the same, 3.8 and 4.0 gm. The loss of vitamin B in the sulfured products was entirely comparable by rat and pigeon tests in all products, except F2 and F3, the dried sulfured Kadotas. These samples appeared to be considerably better sources of the vitamin in the pigeon than in the rat tests. As was noted previously with raisins (Morgan et al., '35), greater loss of antineuritic activity with SO_2 treatment is seen when rats are used instead of pigeons as test animals. This may mean that another B vitamin, possibly B_4, is destroyed to a greater extent by the treatment than is B_1.

VITAMIN G RESULTS

The amounts of figs fed rats on a vitamin G-free basal diet proved for the most part too small to give clear cut results. The rice polish concentrate previously mentioned was given as source of vitamin B in amount of 0.05 gm. daily. All the fig products when fed at 0.5 and 1.0 gm. levels increased the growth of the animals slightly and in about the same degree, but too little to be considered significant. However, when 3 gm. doses of the dried products were fed to groups of six rats each for 56 days, a total gain of 20 to 40 gm. was noted. No significant differences among the samples could be said to emerge. The fresh fruit was not fed at this higher level. The tentative conclusion may be drawn that the dried figs contained from 1 to 1½ Sherman units of vitamin G in 3 gm.—a value not affected measurably by sulfuring or other variations in the drying procedure.

The chief choices involved in the drying of fruits appear to be sun-drying versus dehydration and sulfuring versus no sulfuring. If one regards only the effect of these processes upon the vitamin the choice is somewhat complicated by the fact that some vitamins are affected advantageously by one choice and others by the opposite choice. Thus vitamins A and C are best preserved by removal of oxidative hazards, that is by the use of dehydration instead of sun-drying and by the employment of sulfur dioxide. Vitamin B appears to be better protected by quite the opposite conditions, sun-drying and no sulfuring. However, dehydration instead of sun-drying is far less injurious to vitamin B than is sulfuring. Vitamin G is apparently unaffected by any of these processes. Fruits rich in vitamins A and C should, therefore, preferably be dehydrated and possibly also sulfured, those rich in B sun-dried and not sulfured. Mission figs should be dehydrated to preserve their vitamin A value, but Kadotas, Calimyrnas and Adriatics, poor in A, should be sun-dried and not sulfured in order to retain their good vitamin B value.

SUMMARY

1. Kadota and Calimyrna fresh figs, preserved in frozen storage, were found to contain vitamin C in measurable amounts, similar to that found in grapes and apricots, but less than that in fresh peaches and fresh prunes. Mission and Adriatic figs contained but little of this vitamin.

None of the dried figs, sulfured or unsulfured, sun-dried or dehydrated, retained measurable antiscorbutic value.

2. The Black mission figs contain considerably more vitamin A precursor than do any of the light colored figs, 100 International units per 100 gm. fresh as compared with 50 in fresh Kadotas and less in the Calimyrnas. The sulfuring process proved favorable to the retention of vitamin A of the Missions and Calimyrnas, but not of the Adriatics. The one de-

hydrated product, Calimyrnas, H12, was far superior to all the sun-dried samples, as was previously found true of dehydrated raisins and other dried fruits. The dried figs contained 50 to 143 International units of vitamin A per 100 gm.

3. Vitamin B(B_1) tested by the rat growth method appeared to be unfavorably affected by sulfuring preceding sun-drying. Fresh Kadotas and Adriatics contained about equal amounts of the vitamin, 25 International or 35 Sherman units per 100 gm. The unsulfured dried Adriatics retained 61 per cent of this and all sulfured products 37 per cent or less, that is 71 and 33 Sherman units per 100 gm. of dried fruit.

By pigeon maintenance test much the same relations were discovered, except that the destruction of the vitamin in the sulfured fruit was less obvious. This may be due to more complete destruction of a third B vitamin needed by rats and not by pigeons.

4. Vitamin G is present in figs, the dried products containing practically the same amount in all cases, 33 to 50 Sherman units per 100 gm. None of the variables of the drying process appeared to affect this vitamin.

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THE VITAMIN B AND G CONTENT OF PRUNES

AGNES FAY MORGAN, MARION J. HUNT AND MILDRED SQUIER

Laboratory of Household Science, University of California, Berkeley

ONE CHART AND ONE FIGURE

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The problem of the effect of various methods of drying upon the vitamin content of fruit has been studied during several years in this laboratory. Certain conclusions have been drawn as to the protective value of dehydration instead of sun-drying, and of sulfuring upon vitamins C and A in peaches, prunes and apricots (Morgan and Field, '29, '30; Morgan, Field and Nichols, '31). No attempt was made until recently to assess the vitamin $B(B_1)$ and $G(B_2)$ value of these dried fruits. The present report presents the result of such determinations upon samples of California prunes.

Eddy, Gurin and Kohman ('32) tested canned prunes, both the French and Italian varieties for vitamins B and G. Their results showed very little content of B in their samples—a fact which cannot be ascribed properly to lack of this factor in the fruit, since the canning process is known to decrease antineuritic value. They made no tests upon corresponding fresh fruit. The vitamin G activity shown by their canned specimens was comparable with that here reported for the dried fruit. The only other report upon the B content of prunes is that made by Osborne and Mendel ('20) upon the B complex. Their limited data apparently indicate nearly normal growth of rats on 2 to 5 gm. of prune per day—a better performance than was obtained on any of the other fruits which they examined.

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The prunes used in the work here reported were purchased in the open market, there being no specification mentioned, except that they be of California origin and of a medium size, fifty to sixty per pound. Several different 25-pound boxes were purchased and no attempt was made to segregate the results obtained with these different lots. They were kept in a cool dry place in the laboratory. The fruit contained 22 to 25 per cent water.

Methods. In general, the technic described by Chase and Sherman ('31) was followed for the vitamin B determinations on rats and that of Bourquin and Sherman ('31) for the vitamin G assay.

The vitamin G tests. The source of vitamin B used in the vitamin G tests was not that recommended by Chase and Sherman, but was a potent rice polish extract made according to the technic of Evans and Lepkovsky ('31). This preparation was found to be rich in the antineuritic property and when made from the polish only, without the rice bran, was almost devoid of vitamin G. Daily doses of 0.05 cc. were found sufficient for practically normal growth when vitamin G was supplied.

Both 28- and 56-day feeding periods were used with results indicating that either period is acceptable, but that, as might be expected, the growth increments per week found in the shorter time are greater than the corresponding values for the longer period. The ratios of the increments produced by the various doses, however, are nearly the same in the 28- and 56-day tests. Some general agreement as to the length of the test period to be used for vitamin G assays is urgently needed in order to make the data from different laboratories comparable.

As may be noted in table 1 and chart 1, the growth increments produced by increasing daily allowances of prunes are not arithmetically proportional to these allowances. Moreover, the rats do not die within the longer experimental period of 56 days when confined to the vitamin G-free diet nor does their weight decline below the initial figure. The

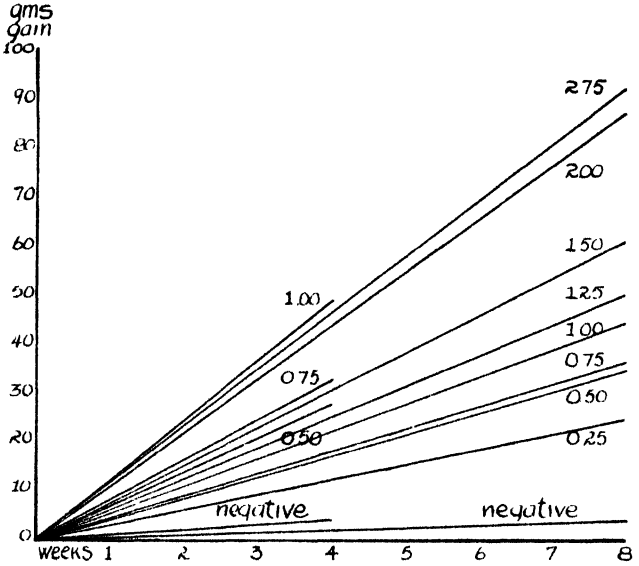

Chart I Gains in weight of young rats fed varying amounts of prune as source of vitamin G. The daily doses are given in grams opposite the growth angles.

TABLE 1

Vitamin G content of prunes

DAILY AMOUNT FED	NUMBER OF RATS	BODY WEIGHTS			NUMBER OF RATS	BODY WEIGHTS		
		Initial	Final	Gain in 28 days		Initial (depleted)	Final	Gain in 56 days
gm		*gm*	*gm*	*gm.*		*gm*	*gm*	*gm.*
0.25					19	66	91	25
0.50	16	55	83	28	21	65	100	35
0.75	14	51	84	33	12	59	96	37
1.00	8	63	112	49	13	58	103	45
1.25					15	56	107	51
1.50					14	54	116	62
2.00					16	52	140	88
2.75					4	58	151	93
Negatives	4	59	65	6	6	64	68	4
	3	49	53	4	14	70	76	6
					21	58	63	5

average gain of forty-one such rats in 8 weeks was 5 gm. Severe dermatitis with loss of fur and sometimes regrowth of soft thin hair, nose bleed, tail scale and blindness were seen in nearly all such animals. Several animals were kept for 6 to 15 months on the vitamin G-free diet. Severe convulsions on disturbance were common among these rats after several months on the diet. One pair of littermates was kept for 18 months, one being given only the G-free diet and the other in addition 2 gm. of prune flesh daily. Figure 1 illustrates their appearance after 7 months on the diet.

Since no generally accepted standard is as yet available for vitamin G assays, the results shown in table 1 must be summarized in the units suggested by Bourquin and Sherman ('31), that is, the daily amount which produces an average gain of 25 gm. in 8 weeks. This may be taken as 0.25 gm. of prunes at the least and 0.50 gm. at the most, about 266 units per 100 gm. or 75 units per ounce..

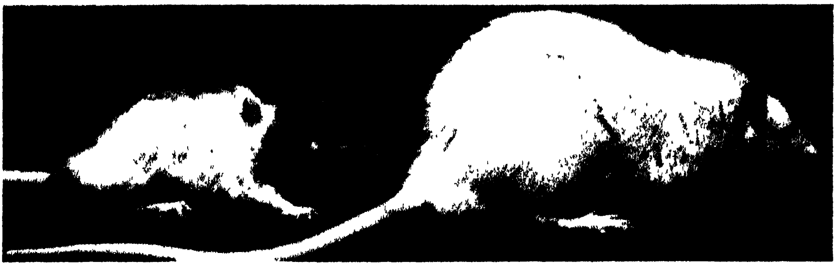

Fig. 1 Prunes as source of vitamin G. Rats of same litter on vitamin G free diet for 7 months, that on the right being given 2 gm. prune flesh daily in addition to basal.

Using the same technic in this laboratory, certain dried foods, reputed to be highly endowed with vitamin G, have been assayed for comparison with the prune results. The data are shown in table 2. It will be noted that prunes are nearly equal to wheat germ and are reasonably close in value to the solids of milk and eggs. The solids of spinach contain two to three times this amount of the vitamin and those of brewery yeast and beef liver about four times as much. No other fruit tested in this laboratory approaches prunes in this respect.

The vitamin B results. Most of the vitamin B tests were made with a basal diet consisting of purified casein 18 per cent, Osborne and Mendel salt mixture 4 per cent, starch 53 per cent, butterfat 8 per cent, cod liver oil 2 per cent, and yeast (autoclaved for 6 hours at 15 pounds' pressure) 15 per cent. In certain cases, however, the yeast was omitted with corresponding increase in starch and a potent liver extract used instead as source of vitamin G. This was done in the hope of discovering whether the laxative effect of the prunes augmented by the similar yeast effect may have resulted in a disproportionate intestinal loss of their vitamin value. The liver extract, which was made and autoclaved under the supervision of Dr. S. Lepkovsky, was shown to be rich in vitamin G and poor in vitamin B. Nevertheless, poorer growth was seen in the rats fed this diet than in com-

TABLE 2

Vitamin G content of certain dried concentrates

FOOD	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN IN 56 DAYS	SHERMAN UNITS PER GRAM
	gm.		*gm.*	*gm.*		
Prunes	0.25	19	66	91	25	4.0
	0.50	21	65	100	35	2.8
Wheat germ	0.25	4	53	85	32	4.1
	0.50	15	65	112	47	3.7
Dried whole eggs	0.50	8	68	124	56	4.5
Dried whole milk	0.25	8	61	96	35	5.6
	0.50	7	77	128	51	4.1
Dried spinach	0.25	8	62	124	62	9.9
	0.50	4	67	170	103	8.2
Dried brewery yeast	0.12	2	74	135	61	19.5
	0.25	8	67	156	89	14.2
Dried beef liver	0.12	4	69	116	47	15.0
	0.25	18	59	158	99	15.8

parable animals given the diet containing autoclaved yeast. As may be seen in tables 3 and 4, the International standard preparation as well as our rice polish concentrate yielded poorer growth when the liver extract was used. Apparently, the vitamin G content of the liver extract allowance (0.4 cc. daily) was insufficient, or some other condition, possibly an effect on appetite, or lack of a third vitamin B factor, interfered with the welfare of these animals.

TABLE 3

Vitamin B content of prunes (with autoclaved yeast as source of vitamin G)

SUBSTANCE	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN	PERIOD
	gm.		*gm.*	*gm.*	*gm.*	*days*
Prunes	0.75	8	49	71	22	56
	1.00	17	63	88	25	56
	1.25	21	62	90	28	56
	1.50	23	56	90	34	56
	2.00	8	58	103	45	56
	2.75	10	62	113	51	56
	0.00	23	50	46	—4	25
Rice polish concentrate	0.025	14	57	100	43	56
	0.050	15	58	160	102	56
	0.075	4	76	213	137	56
	0.100	4	74	221	147	56
International standard	0.005	4	75	67	—8	56
	0.010	18	68	102	34	56
Wheat germ	0.15	9	60	103	43	56
Whole wheat	0.6	9	62	93	31	56
	0.8	6	78	137	59	56

Three different lots of vitamin B acid clay adsorbate used as International standard, and received from Dr. E. M. Nelson, of the United States Department of Agriculture, were tested for growth stimulation. In no case did the unit amount, 10 mg., produce the nearly normal growth expected from the published reports of similar tests by Chick and Jackson ('32) and Kemmerer and Steenbock ('33). However, in both the latter experiments the feeding period was only 4 or 5 instead of 8 weeks. A similar low antineuritic value was noted when the adsorbate was fed to pigeons using the prophylactic method of Salmon ('27).

If our results with the International standard be compared with those obtained with the rice polish concentrate, the latter may be seen (table 3) to contain 1 unit of vitamin B in 0.02 gm. The prunes which we used contained 1 such unit in 1.5 gm. If these values be expressed in Sherman units (the amount allowing 25 gm. gain in rats during 8 weeks), the figures are 0.015 and 1.0 or 1.25 gm. This indicates 22 to 28 Sherman units per ounce or 80 to 100 per 100 gm. The results obtained with wheat germ and whole wheat in a parallel series of tests are quoted in table 3 for comparison and evaluation of the method.

TABLE 4

Vitamin B content of prunes (with liver extract as source of vitamin G)

SUBSTANCE	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN	PERIOD
	gm.		*gm.*	*gm.*	*gm.*	*days*
Prunes	1.00	18	60	51	—9	39
	1.50	10	64	84	20	54
	2.00	21	69	77	8	56
	2.75	8	74	102	28	56
Nothing		9	75	50	—25	18
Rice polish concentrate	0.025	5	61	95	34	56
	0.050	14	66	139	73	56
International standard	0.005	7	73	70	—3	56
	0.010	4	64	88	24	56

SUMMARY

1. The vitamin G(B_2) content of dried California (French) prune flesh is at least 266 Sherman units per 100 gm.. This is larger than has been reported for any other fruit, is comparable with the value found for wheat germ and is one-half to one-fourth that of dried milk, eggs, spinach, yeast and liver.

2. Both 28- and 56-day periods are suitable for vitamin G feeding experiments, but the growth increments obtained with one period are not comparable with those of the other.

3. The vitamin B(B_1) content of the dried prune flesh is 80 to 100 Sherman units per 100 gm. Since the International standard adsorbate in our hands yielded only 34 gm. gain in 8 weeks per 10 mg. (1 unit), the prunes may be said to contain 1 International vitamin B unit in 1.5 gm. This is approximately one-tenth the amount found in wheat germ and nearly one-half that of whole wheat.

4. A liver extract substituted as source of vitamin G(B_2) for the autoclaved yeast in some of the B_1 tests allowed less growth than did the yeast-containing diet, although its vitamin G content appeared adequate. Possibly a third B factor is involved in this difference.

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THE RELATION BETWEEN THE BASAL METABOLISM AND THE ENDOGENOUS NITROGEN METABOLISM, WITH PARTICULAR REFERENCE TO THE ESTIMATION OF THE MAINTENANCE REQUIREMENT OF PROTEIN [1]

D. B. SMUTS [2]

Division of Animal Nutrition, University of Illinois, Urbana

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The energy metabolism of an animal may be reduced to a minimum level by removing the gross influences tending to stimulate heat production, particularly muscular activity, the influence of food, and the stimulus of cold. This basal metabolism represents for each individual the minimum rate of energy expenditure compatible with the maintenance of life. It measures, therefore, the maintenance requirement of energy of the animal, a quota of energy that must be provided by the food consumed if the normal life activities are to be maintained.

Similarly, the nitrogen metabolism may be reduced to a minimum level by removing certain gross influences that tend to stimulate the wastage of nitrogen from the body. In this case, the disturbing influences, while few in number, are somewhat more difficult to remove. Muscular activity is not among these influences, since it does not appreciably affect nitrogen wastage from the body, except as it may induce a negative balance. There are only two requirements for the attainment of a minimum level of nitrogen output, the 'endogenous metabolism' of Folin. The first is that the protein intake should be reduced preferably to zero, or at least to such a level, depending upon the character of the dietary protein, that it will exert no appreciable influence on the rate of nitrogen excretion by the kidneys. The second is that the energy intake should be great enough to protect the nitrogenous constituents of the tissues from catabolism in so far as this is possible. Under these conditions, as Folin ('05) first discovered, the nitrogen output in the urine is reduced to a very low level, which may be considered to measure in a very true sense the requirement of nitrogen (protein) by the animal for the maintenance of life,[3] specifically for the maintenance of the nitrogenous integrity of the tissues (Mitchell, '26, '29; McClellan and Hannon, '32). Folin further showed that, on a creatine-free diet, the creatinine output in the urine is unaffected by the protein intake, and hence presumably that creatinine is a product only of the endogenous nitrogen metabolism.

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[1] The experimental data reported in this article have been taken from the thesis of D. B. Smuts submitted in 1932 to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Husbandry. This paper was prepared for publication by H. H. Mitchell.

[2] Holder of the Webb Stipendium of the University of Stellenbosch, Union of South Africa, 1930–1932.

It seems reasonable to believe that there is a relationship, directly or indirectly causal in nature, between the minimal catabolism of the nitrogenous constituents of protoplasm and the minimum rate at which energy is liberated, as measured by the basal heat production. Both represent the 'idling speed' of the organism, one with reference to the catabolism of a particular class of cellular constituents characterized by their content of nitrogen, the other with reference to the total catabolism of the organism. The total energy liberation of the 'idling' organism includes the energy liberated in those chemical reactions constituting the endogenous catabolism and yielding ultimately the end-products of this catabolism. From this fact only one would expect a measurable correlation between the two, the size of which would depend upon the fraction of the total energy transformations represented in the energy liberated by these endogenous reactions involving the nitrogenous constituents of the tissues.

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[3] This interpretation of the 'endogenous metabolism' is quite in accordance with the original conception of Folin. In this paper, as in the early studies of McCollum and Steenbock ('12) on the pig, the endogenous level of protein metabolism is taken as that level reached on a nitrogen-free diet when consumed in amounts to cover the energy requirements of the animal. If this criterion of the attainment of the endogenous protein metabolism differs at all from that of Folin, it is in the inclusion of a certain amount of protein metabolism of the exogenous type, but the inclusion of such a fraction seems inherently improbable, since with the rat this level of nitrogen excretion is not depressed by an increase in the consumption of nitrogen-free food, if the intake of food energy is slightly above the requirement of maintenance (Mitchell, '24). That nitrogen equilibrium may be attained on an intake of digestible nitrogen—this term being used in the ordinary sense—just sufficient to cover the endogenous losses in the urine, has been shown by McCollum and Steenbock ('12, p. 61) for the pig, using corn or oat protein, and by Mitchell ('24, p. 889) for the rat, using the mixed proteins of milk and lactalbumin.

Only a few investigations have been concerned directly with the relation existing between the basal metabolism and the endogenous nitrogen metabolism. In 1914, Palmer, Means and Gamble studied the relation between basal metabolism and creatinine elimination, with the expressed hope of finding a better basis for the comparison (and prediction) of the basal metabolism of different individuals than that afforded by body weight or body surface. For eight men subjects the average number of calories per milligram of creatinine excreted was 0.98, and for nine women subjects it was 1.26. Although the men varied in weight from 58 to 94 kg., the individual ratios were remarkably constant, possessing a coefficient of variation of only 4.7. The women subjects varied from 48 to 80 kg. in weight and their ratios varied to the extent of 5.2 per cent.

If one may assume that the creatinine elimination is a function of the protein content of the body, then the work of Palmer, Means and Gamble receives confirmation from the investigations of Kohlrausch ('29–'30) concerned with methods for the determination in vivo of the chemical constituents of the body. Kohlrausch experimented with four dogs, varying in weight from 3.8 to 11.5 kg., some of which had been trained to develop their muscular tissue, while others were untrained. By determination of the basal metabolism and actual analysis of their carcasses, he found a constant relationship between the former and the protein content, such that the basal heat per minute (in calories) divided by the two-thirds power of the weight of protein in grams equaled 2.30.

Terroine and Garot ('26) applied to eight species of animals (rat, guinea pig, rabbit, dog, sheep, man, horse and cow) the same type of investigation as that of Palmer, Means and Gamble. They found, however, that the ratio of creatinine elimination (in milligrams) to basal heat (in calories × 100) was not constant, but varied from 29 for the rat to 135 for the horse. Hence, while the ratio may be constant for any one species, it is apparently not constant between species.

The most direct attempt that has heretofore been made to establish a relationship between the basal heat production and the output of endogenous nitrogen has been made in the laboratory of Terroine at Strassburg. Terroine designates the sum of the urinary and fecal nitrogen on a nitrogen-free diet as the total endogenous nitrogen. His basal heat determinations were made by the Haldane gravimetric method (1892), and generally extended over several hours, during which minimal activity, but not complete repose, prevailed. In the large majority of cases, if not in all, the two determinations were not made upon the same animal, the basal heat generally being computed from the numerous determinations reported from previously published experimental data.

In the first paper of this series, Terroine and Sorg-Matter ('27) reported average ratios on three mice, six rats, three pigeons, three chickens and three rabbits, and computed ratios for man and the dog. These specific ratios ranged from 2.30 to 2.90 mg. of endogenous nitrogen per calorie of basal heat, averaging 2.61 mg. Their coefficient of variation is 9.4.

In a second paper by the same authors ('28), the ratio of endogenous nitrogen to basal heat was studied with a number of mice and pigeons exposed to different environmental temperatures, some below the critical temperature and some above. It was found at the lower temperatures that the elimination of endogenous nitrogen increased as well as the basal metabolism and somewhat in the same proportion, so that the ratio of the two was not greatly disturbed. However, in these experiments the ratios were much lower than in the first experiment, averaging 2.18 to 1, the range being from 1.97 to 2.45. No food intakes are reported, so that one is unable to determine whether the observed increases in the total nitrogen excretion were associated with adequate food intakes, or whether they included an increment in nitrogen metabolism induced by an inadequate intake of food energy.

In a third paper by Sorg-Matter ('28), three rats and nine roosters of varying weights and ages were studied. Again the endogenous nitrogen per calorie of basal heat fell within a small range, 2.20 to 2.53 mg., averaging 2.37 mg. Apparently, the more intense basal metabolism of the young animal is associated with a more intense endogenous nitrogen metabolism. The same sort of study on two pigs at widely different body weights was later reported by Terroine, Boy, Champagne and Mourot ('33). The endogenous nitrogen elimination of the pigs (in urine only) was measured at weights of 13 to 14 kg. and again at a weight of 132 kg. The weights of urinary nitrogen per calorie of basal heat varied from 1.8 to 2.0 mg. The fecal nitrogen was determined only at the heavier weights, and at these weights the total endogenous excretions (urine and feces) per calorie of basal heat were 2.3 and 2.1 mg. Summarizing all of their work in this article, it is concluded that the "law of the constant relationship of minimal nitrogen and energy output" holds for the whole series of homeotherms from mouse to pig, with body weights ranging from 1 to 8800 and heat production per kilogram of body weight ranging from 1 to 40.

Ashworth and Brody ('33 a and '33 b) have also published the results of some studies on the relation existing between the basal energy metabolism of rats and their minimum excretion of endogenous nitrogen. With different rats they report, contrary to Terroine, a very variable ratio, ranging from about 0.7 to 2.0 mg. of endogenous nitrogen per calorie of basal heat, and averaging about 1.4 mg. No effect of age on this ratio is evident in their work, either from a group of rats upon which both determinations were made, or from another group for which the basal metabolism was estimated.

The subject of Terroine's investigations is one of scientific importance, since it attempts to associate causally two basic phases of animal metabolism hitherto considered separate and distinct. Incidentally it confirms strikingly Folin's conception of the endogenous nitrogen metabolism, the constancy, or even the reality, of which has been questioned by some investigators. Furthermore, the subject possesses practical significance. The basal metabolism of animals measures their basal (or maintenance) requirement for food energy, while the endogenous loss of nitrogen measures their basal (or maintenance) requirement for nitrogen. The basal metabolism can be measured satisfactorily if the proper equipment is available, but the endogenous nitrogen elimination is difficult to measure, except for small laboratory animals and for pigs, because of the difficulties involved in compounding and feeding suitable rations. If there is a constant relation between the two, it should be possible to estimate the endogenous nitrogen (and the maintenance requirement of protein) of any animal from its basal metabolism, as measured, or as estimated, from body size.

However, Terroine's data are not completely satisfactory, for the following reasons:

1. The inclusion of fecal nitrogen with urinary nitrogen combines two factors of different antecedents. The urinary nitrogen on a nitrogen-free diet is related to body size, and, above a certain minimum intake, is not related to food intake (Mitchell, '24). The metabolic nitrogen of the feces, however, though of body origin, is mostly related to the processes of digestion and varies directly with the amount of digestive work done, as measured roughly by the dry matter consumed. Only a small fraction of the metabolic nitrogen of the feces of rats is unrelated to the digestion on normal intake of food (Schneider, '34). Hence, the total endogenous nitrogen of Terroine, including both fecal and urinary nitrogen, will vary

in size directly with the intake of food, although the true endogenous nitrogen, in the sense of Folin, is not so affected.

2. Terroine, in practically all of his work, determined the endogenous nitrogen on each experimental animal, but estimated the basal metabolism from average values obtained on other animals of the same species and in some cases in other laboratories. However, individual differences, both with regard to basal metabolism and to endogenous nitrogen, have been observed and are undoubtedly factual. The use of an observed value for one factor and an estimated value for the other will obscure the relationship that may exist between the two, so that the ratios obtained will exhibit a fictitious variability.

3. The basal metabolism determinations of Terroine and Trautmann, which were used in many of the calculations, were not made under conditions of absolute repose of the experimental animals. They are, therefore, to some extent too high and too variable.

4. The observed ratios of endogenous nitrogen to basal calories, while suggestive of a constant relationship, exhibit considerable variation, particularly as between different sets of data reported in different publications. The data of Ashworth and Brody are even more at variance with the conception of a constant relationship.

The following investigations were inspired by the work of Terroine and colleagues, but were carried out with the above criticisms in mind. It was hoped, by correcting what appeared to be defects in the experimental procedures and technic of Terroine, to secure more convincing evidence of a constant relationship between the endogenous nitrogen elimination of animals of different species and their basal metabolism. Such evidence has, the writer believes, been secured.

PLAN OF EXPERIMENT

The experiments were carried out upon five species of animals, the mouse, rat, guinea pig, rabbit and pig. The mice ranged in weight from 20 to 30 gm., averaging 24, and included two females and seven males. Of the twenty-three rats in the experiment, fifteen were males and eight females. The former averaged 243 gm. in weight (202 to 318), and the latter 186 gm. (170 to 227). Of the nine guinea pigs, six were males; the body weights averaged 414 gm. (300 to 489). The ten rabbits were all males and ranged in weight from 1440 to 2740 gm., averaging 2072 gm. The five pigs also were all of the male sex; their weights were 62.3, 66.7, 68.0, 69.5 and 71.7 kg. Upon each of these fifty-six animals the endogenous nitrogen was determined and at least one basal metabolism measurement was made. To permit expression of the latter measurement in the usual terms of calories per square meter of body surface per day, it was necessary to determine the surface areas of a number of mice, guinea pigs and rabbits, for which species good prediction formulas did not seem to be available, and to devise mathematical equations relating surface area to body weight. The results of this phase of the study have been published elsewhere (Smuts, '33).

The general plan of procedure was, first, to place each animal for at least 7 days upon a low-protein (10 per cent), but otherwise complete diet (table 3). The animal was then fasted for a period of time, varying with the species, until the post-absorptive condition was established. For mice, guinea pigs, rabbits and pigs it was considered necessary, in auxiliary tests, to determine the period of fasting required for this purpose. The basal metabolic rate was then determined.

The animal was next placed upon a low-nitrogen diet until its nitrogen metabolism was reduced to the endogenous level. The length of time required in the various species of animals for the depletion of the so-called 'deposit protein' was studied, and, at the termination of this period, the urine was collected for 7 days and its nitrogen content determined.

Those animals upon which a second basal metabolism determination was to be made were then, after the period of low-nitrogen feeding, put again upon the complete standard diet for 7 days and then fasted as before.

METHODS

Basal metabolism. The basal metabolism of the mice, rats, guinea pigs and rabbits was determined by a modification of the gravimetric method first proposed by Haldane (1892). The respiratory quotient was determined by the ordinary technic in a period of 6 to 8 hours. During this time the animals, although confined in a small chamber, exhibited more or less activity, which is incompatible with a basal level of metabolism. Hence the basal production of CO_2 was determined during short periods (ranging in toto from 40 to 60 minutes) of complete muscular repose. During these periods, the animal was under constant observation, and at the initiation of any appreciable activity the period was interrupted, to be resumed again only when the animal had become quiet and had stayed quiet for at least 5 minutes. The carbon dioxide produced during these truly basal periods was collected in a separate chain of absorption bottles, to which the ventilating current could be diverted by means of a three-way stopcock.

The basal metabolism of the experimental pigs was determined in a pneumatic chamber.[4] The chamber has a volume of 1190 liters. During a test, the air within the chamber was vigorously stirred by an electric fan and its moisture content was kept down by exposure to sticks of P_2O_5 contained in a shallow porcelain dish. Samples of air were taken from the chamber at the beginning and the end of a test and analyzed for CO_2 and O_2. The gaseous exchange of the animal was computed from, a) the difference in composition of the chamber air at standard temperature and pressure; b) the air volume of the chamber, and, c) the elapsed time. The tests generally continued for about 60 minutes, during which time the CO_2 content of the air reached a concentration of about 1 per cent. To prevent any appreciable leakage of air into or out of the chamber, the temperature within the chamber was not allowed to rise above the temperature outside, a result secured by continuously running water over it. What small leakage occurred, was corrected for on the basis of the nitrogen content of the chamber air.

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[4] Grateful acknowledgment is made to the Graduate School of the University of Illinois which provided funds for the construction of this pneumatic chamber. Without this timely aid, it would not have been possible to extend the investigation to pigs.

With pigs the influence of muscular activity upon the heat production could not be as easily removed as with the smaller animals. The only recourse was to discard the measurements obtained during periods of appreciable activity, which was possible, because the pig could be observed through a window in the chamber. The test was then repeated until a period of absolute rest was obtained.

Both methods of determining the basal metabolism neglect protein metabolism, since the total respiratory quotients are used in the calculations as non-protein respiratory quotients. The error introduced by this simplification, which is commonly made in such work, is negligible under basal, and even under most conditions, rarely exceeding 2 per cent.

The significance and reproducibility of a basal metabolism determination depend very largely upon the removal of the stimulus to heat production exerted by the last meal. The experimental rats were fasted for at least 17 hours before the basal measurements were made. On the basis of the work of Benedict and MacLeod ('29 a), it was concluded that postabsorptive conditions prevailed after this time. For the other species of animals, special tests were made to determine the time of removal of the food stimulus.

A study was undertaken with five mice to determine the necessary fasting period, from which it appears that 10 hours are sufficient for the establishment of a constant rate of heat production. After 10 hours of fasting, the R.Q.'s were usually in the neighborhood of 0.72 to 0.73, which constitutes additional evidence that the mouse at this time is catabolizing its own tissues.

A similar investigation with four guinea pigs showed that, under the conditions of feeding imposed, at least 20 to 22 hours of fasting were necessary for the attainment of a true basal condition of metabolism.

The results obtained with three rabbits are interesting. The heat production was determined at twelve hour intervals for periods of 72 or 84 hours, with the results given in table 1. The low rates of heat production for all three rabbits after 24 hours of fast are difficult to understand. A constant level of heat production was obtained only after 60 hours of fast.

TABLE 1

The heat production of rabbits after different periods of fasting

ANIMAL NO.	BODY WEIGHT	TIME FASTED	DAILY HEAT PRODUCTION	HEAT PRODUCTION PER DAY PER M² OF BODY SURFACE	R.Q.
	gm.	*hr.*	*cal.*	*cal.*	
R1	2330	10	206.9	1250	0.88
	2250	24	106.8	655	0.85
	2310	24	105.7	641	0.86
	2240	36	126.6	777	0.83
	2215	36	121.0	747	0.81
	2240	48	112.0	688	0.77
	2170	60	94.2	587	0.74
	2240	72	90.5	556	0.71
	2155	84	102.1	638	0.70
R2	2308	10	198.4	1204	0.90
	2200	24	116.5	722	0.81
	2120	36	152.8	984	0.79
	2100	48	147.4	931	0.79
	2120	60	119.6	755	0.73
	2110	72	118.3	747	0.70
R3	2550	24	125.7	730	0.81
	2500	42	159.0	932	0.77
	2570	66	120.5	698	0.75
	2565	72	122.9	712	0.69

The rate of decline in heat production during fast for two pigs is shown by the values contained in table 2. The decline is very gradual, in spite of the simple structure of the gastrointestinal tract in the pig. Only after 96 hours of fast may the heat production be considered to have reached a fairly constant level. This finding is in agreement with the work of Lenkeit ('31), who reported that only after 4 days had the major portion of a test meal completely traversed the alimentary canal of the pig. Capstick and Wood ('22–'23) found that the metabolism of a pig decreased after feeding for about 90 hours.

Hence, in the routine measurement of the basal metabolism of the animals used in this experiment, the following periods of fast were imposed: mice 10 hours, rats 17 hours, guinea pigs 22 hours, rabbits 60 hours and pigs 96 hours.

To remove the stimulus of cold, which is also incompatible with a basal condition of metabolism, it is necessary to maintain an environmental temperature above a certain minimum, which is known as the 'critical' temperature. The following minimal temperatures were observed throughout this work; the evidence, where available, that these temperatures are critical will be found in the literature articles cited:

TABLE 2

The heat production of pigs after different periods of fasting

TIME FASTED	PIG NO. 4		PIG NO. 5	
	R.Q.	Heat production per m² per hour	R.Q.	Heat production per m² per hour
hr.		*cal.*		*cal.*
4	0.99	74.5	1.20	86.9
24	1.00	85.0	0.83	63.9
48	0.85	51.0	0.79	52.6
72	0.80	51.5	0.78	39.0
96	0.79	45.2	0.80	38.7
120	0.75	42.4	0.78	35.3
144	0.74	44.2	0.79	32.0

Mouse, 29 to 30°C. (Asźodi, '21; Benedict and Fox, '32–33).
Rat, 28°C. (Benedict and McLeod, '29 b).
Guinea pig, 32 to 33°C. (Murschhauser, '12; Rubner, '02).
Rabbit, 27 to 28°C.
Pig, 21°C. (Capstick and Wood, '22; Deighton, '29).

It was assumed that the rabbit, with its ample fur covering, would not exhibit a cold stimulus at a temperature as high as 27 to 28°, though no experimental evidence on this point was obtained from the literature.

The endogenous nitrogen excretion. The low-nitrogen rations used in reducing the experimental animals to the minimum endogenous catabolism are described in table 3. It will be noted that the ration used for pigs contained no considerable source of nitrogen, with the exception of 1 per cent of yeast, while the rations used for the other animals contained approximately 5 per cent of dried ether-extracted whole egg. Mitchell and Carman ('26 c, p. 200) have shown that egg protein at this low level does not affect the output of either fecal or urinary nitrogen, while its presence in the diet maintains the appetite and the weight of experimental rats much

TABLE 3

Percentage composition of the experimental rations

INGREDIENTS	STANDARD RATION	NITROGEN-LOW RATIONS FOR				
		Rats	Guinea pigs	Mice	Rabbits	Pigs
Dried ether-extracted whole egg	....	5.02	5.02	5.02	5.02	....
Casein	12.14	....	....	....	....	....
Salts	4.00	4.00	4.00	4.00	4.50[1]	4.00[2]
Butterfat	10.00	10.00	12.00	10.00	....	....
Cod liver oil	....	....	....	....	2.00	1.00
Mazola oil	....	....	....	....	10.00	....
Sodium chloride	1.00	1.00	1.00	1.00	1.00	....
Sucrose	10.00	10.00	10.00	10.00	10.00	....
Cellu flour[3]	4.00	4.00	20.00	4.00	25.00	....
Cellomass	....	....	....	....	1.00	2.00
Yeast	....	....	....	....	1.00	1.00
Starch	58.86	65.98	47.98	65.98	40.48	92.00
Per cent nitrogen		0.65	0.67	0.65	0.68	0.14
Calories per gram[4]		4.0	4.2	4.0	4.3	4.0

[1] A modified Osborne and Mendel salt mixture, proposed by P. B. Hawk and B. L. Oser, 1931, Science, vol. 74, p. 369.

[2] Salt mixture for pigs. Steamed bone meal 15 parts, ground limestone 15, sodium chloride 15, magnesium carbonate 1.5, potassium carbonate 1.5, potassium sulphate 1.0, ferric chloride 0.75, potassium iodide 0.25.

[3] Cellu flour is a product put out by the Chicago Dietetic Supply House. By actual analysis it contains 37.8 per cent crude fiber, and only 0.015 per cent of nitrogen.

[4] Determined by the Parr oxygen bomb calorimeter.

better than does a practically nitrogen-free diet. The cost of this egg preparation prevented its use in the pig experiment.

The period of time required to attain the endogenous nitrogen excretion will vary with the different species. It has been shown by Mitchell ('24, tables 15 and 16) and again by Mitchell and Carman ('26 c, table 17) that the rat rapidly adjusts itself to its endogenous nitrogen metabolism, only 3 or 4 days being required, and maintains a constant level, biologically speaking, for at least 10 days or 2 weeks. However, in the present investigation the experimental rats were kept for 6 days on the low nitrogen diet before the collection of urine was started.

To determine the necessary period of preliminary feeding for the other species of animals, tests were made upon three representatives of each species, the outcome of which is given in table 4. As a result of these tests, in all subsequent work the following periods of preliminary feeding were adopted: for mice, 5 days; for guinea pigs, 8 days; for rabbits, 15 days; and for pigs, 20 days.

The periods for the collection of urine were in all cases 7 days in length. The animals generally ate well during these tests, but if there was a distinct failure of appetite and a corresponding rapid loss in weight, the animal was discarded.

All of the animals were confined in individual dishes or cages, varying in size with the size of animal, but similar in design. In all cases, the animal rested upon a perforated floor, made of glazed earthenware (rats and mice), hardware cloth of ½ inch mesh (guinea pigs), aluminum alloy bars (rabbits), or heavy reinforced wire fencing of 1 inch mesh (pigs). The feces were readily passed through this floor, except in the case of the pigs, and were caught upon copper window screening suspended between the perforated floor and the bottom of the dish or cage to which the urine drained. In the metabolism receptacles in which the mice, rats and guinea pigs were confined, the urine was absorbed upon filter paper; in the rabbit and pig cages it drained along a sloping surface to a container below. The collections of urine were made daily by the methods employed in this laboratory, the adequacy of which has been satisfactorily demonstrated. The completeness of the collections for rats and mice, about which some investigators are sceptical, was retested in connection with these studies, and percentage recoveries of 97.5 to 99.6 were obtained in nine trials.

TABLE 4

The adjustment of the urinary nitrogen to the endogenous level in mice, guinea pigs, rabbits and pigs. Results expressed in milligrams per day

DAYS ON LOW-NITROGEN RATION	MICE			GUINEA PIGS			RABBITS			PIGS		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
1	15.0	17.8	15.8									
2	14.6	17.4	15.0									
3	13.4	16.9	14.3	146								
4	13.0	16.9	14.5	144								
5	13.1	16.6	13.7	138	124	132						
6	13.3	17.8	14.6	128	123	122						
7		16.6		115	99	115	558			6292	3312	4889
8				111	92	113	436	400		7065	3193	4492
9				111	90	95				6008		5213
10				110	93	110	433		278	4782	3152	4795
11				110	92	93	413	376	270	4876	3053	4762
12						94	385		275	6222	3551	4552
13						91				4398	5452	5237
14						90	255	295	217	4273	4050	
15						98	243	277	200	4050	3431	
16							257	244	220	3936	3727	4172
17							253	243	220	3728	3843	4239
18								250		3727		4300
19								247		3830	2826	4006
20											2745	3962
21										3721	2632	3989
22										2640	2651	3446
23										2846	2741	3843
24										2683	2699	2680
25												
26										2947	3173	3207

All urine collections were analyzed for total nitrogen by the Kjeldahl method, using mercury and potassium sulfate in the digestion mixture, and for total creatinine (including any creatine present) by a modification of the Folin method already described (Mitchell and Carman, '26 c).

THE RESULTS OF THE EXPERIMENTS

The essential results of the experiments, averaged to save space, are given in table 5 together with statistical measures of the variation of the individual data. The average data from the initial basal metabolism determinations[5] are given and related to the average total endogenous nitrogen excretions, the ratios in the last column being obtained from the two individual measures expressed per m^2 of body surface. The average creatinine excretions are given in various forms and, in the last column, the average ratio of the creatinine N to the output of basal heat, both being expressed on the surface area basis.

The basal metabolism determinations of this study may profitably be compared with some of those previously reported on the same species of animals by other investigators, representing what appear to be the best published determinations on animals in the later stages of growth. All values cited below have been computed from surface areas estimated according to the formulas given on page 419. They are hence directly comparable with one another.

For mice, the reported values are extremely variable, probably because of the difficulty of maintaining quietness for any length of time with this species—a point which Benedict and Fox ('32–'33), in particular, emphasize. However, the average basal metabolism rate obtained in this experiment, 1192 calories per m^2 per day, is considerably higher than that of Benedict and Fox, 726 calories, or the closely agreeing values of Davis and van Dyke ('33), 735 calories, and of Aszódi ('21),

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[5] In these basal metabolism determinations, the respiratory quotients averaged 0.72 for mice, rats and guinea pigs, 0.73 for rabbits, and 0.75 for pigs. The individual quotients varied only from 0.70 to 0.75 with two exceptions: for two of the pigs the R.Q. was 0.79.

TABLE 5

Summary of average determinations and ratios for the various species of animals. These results are all expressed on the daily basis

SPECIES	NUMBER OF ANIMALS	BASAL METABOLISM[1]		ENDOGENOUS N[1]		RATIO OF ENDOGENOUS N TO BASAL HEAT PER M[2]	CREATININE NITROGEN[1]			CREATININE COEFFICIENT	RATIO OF CREATININE N TO BASAL HEAT
		Per kilogram body weight	Per m² body surface	Per kilogram body weight	Per m² body surface		Per kilogram body weight	Per m² body surface	Per cent of total endogenous N		
		cal.	*cal.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*mg.*	*mg.*
Mouse	9										
Average		322	1192	614	2308	1.941 ± 0.030	30.1	113	4.92	81	0.0951
Standard deviation		22.1	62.3	45.6	105.8	0.136	2.53	5.14	0.277	....	0.0035
Coefficient of variation		6.9	5.2	7.4	4.6	7.01	8.4	4.5	5.6	....	3.69
Rat	23										
Average		109	748	213	1481	1.988 ± 0.023	15.1	95.5	7.11	41	0.141
Standard deviation		14.6	88.3	22.1	156	0.161	1.50	14.0	0.800	....	0.0176
Coefficient of variation		13.4	11.8	10.4	10.5	8.10	10.0	13.3	11.2	....	12.48
Guinea pig	9										
Average		95	958	178	1827	1.908 ± 0.034	12.7	131	7.18	34	0.137
Standard deviation		7.21	50.1	14.4	160	0.150	0.61	10.0	0.720	....	0.0125
Coefficient of variation		7.6	5.2	8.1	8.8	7.86	4.8	7.6	10.0	....	9.09
Rabbit	10										
Average		59	777	121	1652	2.128 ± 0.019	17.1	233	14.3	46	0.304
Standard deviation		7.06	115	13.9	256	0.091	1.54	30.8	2.5	....	0.0522
Coefficient of variation		12.0	14.8	11.5	15.5	4.26	9.0	13.2	17.5	....	17.17
Pig	5										
Average		19.6	959	40.4	2006	2.100 ±0.038	7.3	360	18.2	19.5	0.378
Standard deviation		1.85	96	3.6	187	0.128	1.1	60	3.8	....	0.063
Coefficient of variation		9.4	10.0	8.8	9.3	6.10	15.5	16.8	20.9	....	16.69
Grand average						**2.004 ± 0.015**					

[1] Surface areas were estimated from body weights in this table by the following formulas:

Mice	$S_{cm^2} = 15.18\ W_{gm.}^{.456}$ (Smuts, '33)
Rats	$S_{cm^2} = 12.54\ W_{gm.}^{.60}$ (Lee, '29, Am. J. Physiol., vol. 89, p. 24)
Guinea pigs	$S_{cm^2} = 7.35\ W_{gm.}^{.667}$ (Smuts, '33)
Rabbits	$S_{cm^2} = 56.33\ W_{gm.}^{.490}$ (Smuts, '33)
Pigs	$S_{m^2} = 0.097\ W_{kg.}^{.633}$ (Brody, '28, Mo. Agr. Exp. Sta. Res. Bull no. 115)

746 calories. This is the more puzzling, since none of these investigators claims that his animals were completely quiescent, while in this study the experimental mice were observed to be at absolute rest. A possible explanation is the apparent instability of the temperature-regulating mechanism of the mouse—a phenomenon described in considerable detail by Asźodi.

The basal metabolism values for rats obtained in this experiment, averaging 748 calories per m² per day, were quite in line with previously reported values, but show no appreciable sex difference. Thus, Mitchall and Carman ('26 b) have reported results on forty-two rats averaging 777 and 731 calories per m² for male and female rats, respectively, while values of Benedict and MacLeod ('29 b) on seventeen rats average 798 and 741 calories, respectively. The values for guinea pigs, averaging 958 calories per m², agree well with the results of Murschhauser and Hidding ('12) on four animals, averaging 902 calories, but are somewhat higher than the average reported briefly (148 animals) by Siebert and Thurston ('31–'32), i.e., 856 calories. This is the lowest average value reported in the literature; it seems possible that the subjection of the experimental animals in these studies to a basal metabolism test three to four times a week may have depressed the value to subnormal levels.

The conditions for the determination of the basal metabolism of rabbits have never been defined, in so far as the author is aware. None of the published results of such work specifies the environmental temperature imposed, and none but that of Wiegner and Ghoneim ('30–'31) can, in view of the tests on this point described above, be considered to be truly post-absorptive values. The average value of the ten secured in this experiment, 777 calories per m², approximates that of Wiegner and Ghoneim, 728 calories. That it also approximates other results which were obtained after much shorter periods of fasting, may be traced to the peculiar fact noted above, namely, that a level of metabolism similar to the true post-absorptive level is reached after only 24 hours of fast and possibly somewhat earlier.

The basal metabolism of the five pigs in this experiment averaged 959 calories per m² per day—a somewhat lower value than any previously reported, although it approximates the one result of Capstick and Wood ('22), 1027 calories, and the later results of Deighton ('29) on two pigs, averaging 1079 calories. From Brody's work ('32 b, table 8) female pigs, weighing 75 pounds or more, were found to have basal metabolic rates averaging 1026 calories per m² per day. There may be some question whether these values represent the true post-absorptive conditions, because of the shortness of the fasting period.

The plan of the experiment contemplated a second basal metabolism determination upon all animals at the conclusion of the period of low nitrogen feeding, but a complete second series of such measurements was made only in the case of the male rats and the guinea pigs. For the rats, the second series of results checked well with the first (1 per cent average difference), indicating that the basal metabolic rate was not disturbed by this abnormal dietary regime. For the guinea pigs, however, a consistent rise in basal metabolism was observed, averaging about 10 per cent.

For most animals, published measurements of the endogenous nitrogen output are few, if any at all have been reported, and in this connection the many results of Terroine and associates cannot, unfortunately, be used, because his so-called 'endogenous nitrogen' includes both urinary and fecal nitrogen on a nitrogen-free diet.

From a compilation of 450 determinations of the endogenous nitrogen of rats, mainly from the Division of Animal Nutrition of the University of Illinois, but also from other laboratories in this country and Europe, an average value of 1238 mg. of nitrogen per m² of body surface was computed. The rats varied in weight from less than 30 gm. to 450 gm. There was an apparent tendency for this ratio to be somewhat higher for the heavier rats, averaging about 1430 mg. for rats weighing 150 gm. or more. This value is very close to the average obtained in this study of 1481 mg. for both male and female rats. Ashworth and Brody's voluminous data ('33 a) on the endogenous nitrogen excretion of rats were obtained intermittently during prolonged periods of feeding on low-nitrogen diets. Such data in their entirety are not comparable with the data reported in this paper, since it is probable that the excretion of nitrogen in the urine after such prolonged feeding of this character does not represent the endogenous level of nitrogen metabolism any more than the heat production after prolonged fasting represents the basal metabolism. Hence, the low levels of nitrogen output reported by the Missouri investigators, averaging only about 1000 mg. per m^2 of body surface, are not surprising in view of the long periods of specific nitrogen starvation. Unfortunately, in the tables presenting their data, no information concerning the time at which ration changes were made is given, thus precluding the selection of such of their data as were obtained under conditions comparable to those adopted in this study.

The average excretion of endogenous nitrogen found for the rabbit, 1652 mg. per m^2 of body surface, is higher than the few determinations of this quantity reported in the literature. Mendel and Rose ('11–'12) fed one rabbit on sugar for a sufficient period of time to attain a constant level of nitrogen output, averaging 1466 mg. per m^2 of body surface. A somewhat similar study was conducted by Myers and Fine ('13) on one rabbit. During a 7-week period of carbohydrate feeding, the total urinary nitrogen output averaged 1276 mg. per m^2 of body surface. In a report by Serio ('23), data are given on the urinary nitrogen output of a rabbit during a period of low-nitrogen feeding. For the last 6 days of this period the excretion averaged 1313 mg. per m^2. Although these three results on the rabbit are less than the average excretion obtained for the ten rabbits in this study, they fall essentially within the range of the individual results, i.e., 1278 to 2015 mg.

The average of 2006 mg. of endogenous nitrogen per m^2 of body surface for five pigs may be compared with an average of 1844 mg. for five pigs of similar weight from experiments of McCollum and Steenbock ('12) and McCollum and Hoagland ('13), and an average of 2297 mg. for eight pigs from the experiments of Mitchell and Kick ('27).

Determinations of the endogenous urinary nitrogen excretions for the mouse and guinea pig, with which to compare those made in this study, have not been found in the literature. Such comparisons as have just been made are of value in judging the validity of the relations between basal metabolism and endogenous nitrogen metabolism that will be discussed in the next section of this report.

DISCUSSION OF EXPERIMENTAL RESULTS

From the averaged data of table 5, it is evident that both the basal metabolism and the endogenous nitrogen excretion are much more closely related to body surface than to body weight, since, when expressed to the unit of body surface, much less variable values are obtained among the different species than when expressed to the unit of body weight. Also, it is evident that a marked correlation exists between the two measurements, so that the ratio of endogenous nitrogen in milligrams to basal calories is remarkably constant among the five species of animals studied. The average ratio is approximately 2.00 mg. of endogenous nitrogen excreted for every calorie of basal heat produced. While the differences in average ratio between some of the species, namely, between rabbits and rats, mice, or guinea pigs, and between pigs and guinea pigs or mice, are statistically significant, this fact is due more to the great constancy of the ratio within each species rather than to any great disparity between average specific ratios.

The variability of the ratio within each species is well measured by the coefficient of variation. These coefficients are 7.01 for mice, 8.10 for rats, 7.86 for guinea pigs, 4.26 for rabbits and 6.10 for pigs. These degrees of variability may be compared, for example, with the variability in the basal heat production of adult human beings, expressed per m^2 of body surface. As calculated by Harris and Benedict ('19, p. 147) for different series of observations, the coefficient of variation ranges from 6.75 to 11.13, except for a small series of measurements on athletes, for which the coefficient of variation was 3.97.

The most striking confirmation of the close correlation existing between the basal metabolic rate and the endogenous excretion of nitrogen is afforded by the data on rats and rabbits. The animals of these two species selected for the experiment were in quite different nutritive states, some having been taken from other experiments—this being true of all of the rabbits—in which different conditions were imposed, and some having come directly from the stock colony. As a result, they were the most variable groups, both with reference to the basal metabolism and to the excretion of endogenous nitrogen, as the coefficients of variation (table 5) indicate. But in spite of this great variability in these two factors among the rats and the rabbits, the ratio between them was not unusually variable; in fact, for the rabbits the ratio was more nearly constant than for any other species.

The percentage deviations of the individual ratios of endogenous nitrogen to basal calories from the average ratio for all animals, 2.00, average 6.86. Seventy-seven per cent of these percentage deviations are no greater than 10, and 93 per cent of them are no greater than 15. The coefficient of variation of the individual ratios about the mean of all is 8.12. The ratio of endogenous nitrogen to basal metabolism thus appears to be as constant within these five species of animals as the ratio of basal metabolism to body surface is within the human species, and far more constant than the latter ratio among different species.

The applicability of this very constant ratio of 2 mg. of endogenous nitrogen for each calorie of basal heat to the human species may be tested from the data of two investigations in which the experimental subject was submitted to both measurements. Smith ('26), in his study of the endogenous nitrogen metabolism, also measured the basal metabolism of his subject twice during the period of low-nitrogen feeding. The first determination was made on the thirteenth day of this regime, on which day the subject was producing 1383 basal calories and excreting 2880 mg. of urinary nitrogen, representing a ratio of 2.08 mg. of nitrogen per calorie. At this time, contrary to the experience of Folin and others, the urinary nitrogen had not yet reached a constant level, and shortly after this time its rate of excretion diminished rapidly to the twenty-fifth day of low-nitrogen feeding, when the second basal metabolism determination was made. The ratio of endogenous nitrogen to basal heat now was only 1 mg. per calorie. Two years later, Deuel, Sandiford, Sandiford and Boothby ('28) published their study of the nitrogen minimum in man, throughout which frequent determinations of basal metabolism were made. The first period of this experiment consisted of 30 days of low-nitrogen feeding, and is the only period that need be considered in this connection, since in the later periods conditions were imposed that would be expected to induce rates of excretion of urinary nitrogen bearing no known relation to Folin's endogenous metabolism. On the ninth day of specific nitrogen starvation, the urinary nitrogen excretion had attained an approximate level, and the ratio of this excretion to the basal heat was 1.96 mg. per calorie. For the next period of 7 days, the ratio was 2.21, and for the next period of 6 days it was 1.95. After this time the ratio decreased to 1.44 mg. per calorie.

These two experiments, and particularly the latter, constituting the only published evidence known to the writer, indicate that in the human species, as in the lower animals, the endogenous nitrogen output bears a ratio to the basal heat approximating 2.0 mg. to the calorie. They also indicate that protracted feeding on low-nitrogen diets may disturb this ratio by depressing the endogenous nitrogen output, just as protracted fasting depresses the basal metabolism. Possibly the erratic and generally low ratios reported for rats by Ashworth and Brody ('33 a) are traceable to the variable, but generally long periods of low-nitrogen feeding.

The relation between the creatinine excretion and the basal metabolism for the fifty-six animals of this experiment is also set forth in table 5. Within any one species the ratio of creatinine nitrogen to basal calories is fairly constant, as Palmer, Means and Gamble ('14) found for humans, this being particularly true for mice, rats and guinea pigs. But among the different species, the average ratios increase in size with size of animal, being 0.095 mg. per calorie for mice, 0.141 mg. for rats, 0.137 mg. for guinea pigs, 0.304 mg. for rabbits, and 0.378 mg. for pigs, this being in general agreement with the experimental results of Terroine and Garot ('26). Among rats a distinct sex effect is evident, the ratios of the males being distinctly higher than those of the females, the averages being, respectively, 0.148 and 0.129 mg. of creatinine nitrogen per calorie of basal heat.

The inconstancy of the ratio of creatinine to basal heat in interspecific comparisons, in spite of the constancy of the ratio of total endogenous nitrogen to basal heat, is the result of specific differences in the percentage of endogenous nitrogen represented in the creatinine excretion. This percentage is low for the mouse, 4.92, intermediate for the rat and guinea pig, 7.11 and 7.18, and high for the rabbit and pig, 14.3 and 18.2, showing a positive correlation with body size. In seeking an explanation of these interesting relationships, reference may be made to the data collected and compiled by Grafe, Reinwein and Singer ('25), with reference to tissue respiration when in situ and when removed from the body. From their own data and from data compiled from the literature, they show that similar tissues from different warm blooded animals respire at about the same rate (expressed as oxygen consumed per gram of dry matter per minute) when removed from the body, although in situ they respire at markedly different rates in rough accordance with the surface area law. Furthermore, only in the small animals, such as the mouse, is the rate of respiration of the intact tissue as great as that of the excised tissue. The larger the animal, the greater the depression of tissue respiration with reference to that of the isolated tissue.

From the results of this experiment one may reasonably infer that there is in all warm blooded animals a constant (in the biological sense) ratio between the basal tissue respiration and the basal catabolism of the nitrogen-containing tissue constituents. In small animals, such as the mouse, in which the tissue respiration is the same whether in situ or isolated, the metabolism of creatine accounts for only a small fraction of the total endogenous nitrogen metabolism. In larger animals, in which the rate of tissue respiration in situ becomes increasing smaller than that of the excised tissue, the creatine metabolism assumes a larger and larger role in the total endogenous nitrogen metabolism. In other words, the depression of tissue oxidation in large animals, brought about by some unknown regulatory mechanism the operation of which results in approximate conformance to the surface area law, is accompanied by a parallel depression of the endogenous catabolism, but the greater burden of this latter depression is borne by the non-creatine constituents of the tissues. Whether this means a greater depression of respiration in the non-muscular tissues than in the muscular tissues is an interesting question.

The results of this investigation, together with the work of Terroine and associates, afford a promising method of estimating the maintenance requirements of protein for different animals from the basal metabolism. According to this method, 2 mg. of nitrogen, or 12.5 mg. of conventional protein, are required daily for each calorie of basal heat. Since basal metabolism varies with body surface rather than with body weight itself, it may be concluded that the protein requirements for maintenance vary likewise, and that the current method of expressing such requirements per unit of body weight is definitely in error. While the investigation reported here was concerned mainly with adult animals, Sorg-Matter ('28) found that age did not disturb the relationship between endogenous nitrogen and basal heat, so that, until evidence to the contrary is forthcoming, it seems justifiable to apply the above ratio to warm blooded animals of all ages.

Brody ('32 a) has shown that the basal metabolism of mature mammals and of the domestic fowl may be predicted by the following equation

$$Q = 70.4\, m^{.734}$$

where Q is the basal heat in calories per 24 hours and m is the body weight in kilograms. This equation may be adapted to a determination of the protein requirements by the use of the ratio of 12.5 mg. of protein per calorie of basal heat. The equation then becomes

$$P = .88\, m^{.734}$$

where P is the day's requirement of protein expressed in grams.

It should be understood that this expression of the protein requirement for maintenance refers not to dietary protein, nor to digestible protein, but to protein actually utilized in the replacement of endogenous losses. The corresponding amount of dietary protein would be larger than this, and would be greater, the greater its wastage in metabolism and hence the smaller its biological value. A biological value of 50 may be regarded as a safe one to use in estimating what may be considered an average requirement of digestible protein. Hence, the net requirement of protein, as computed from the above equation, when multiplied by 2 will give an estimate of the requirement of digestible protein of less than average quality. In fact, when even poor protein mixtures are fed at the low level required in a maintenance ration, they should be much better utilized than 50 per cent, so that the margin of safety in this proposed estimate of the maintenance requirement of protein seems ample.

How do the requirements computed in this manner compare with the requirements estimated by the methods of Sherman ('20) and of Armsby ('17)? These methods agree in assigning 0.6 part of digestible protein per 1000 parts of live weight, the Sherman method relating only to humans, while Armsby's method relates to domesticated farm animals of varied sizes. The comparison is given in table 6 for animals ranging in size from 100 gm. to 681 kg.

It is evident that for animals heavier than about 150 pounds, the proposed method gives lower estimates than the other methods. At 150 pounds the estimates are very nearly the same, and at lower weights, the method here proposed gives higher estimates. The lower proposed estimates for the heavier animals is in line with the well-known liberality of the Armsby standard for such animals as beef cattle and dairy cattle, while the higher proposed estimates for very small animals seem obviously nearer the truth. For example, a 200 gm. rat has a basal metabolism of 22.5 calories daily and would require for maintenance of body weight approximately 30 calories of gross food energy (Mitchell and Carman, '26 a) daily, or 7 gm. of food with an energy value of 4.25 calories per gram (also, Mitchell, '24, p. 880). If this ration contains 4 per cent of a high quality protein, such as egg protein, it will slightly more than support nitrogen equilibrium, according to the experience of this laboratory. Seven grams of such a ration will contain 280 mg. of protein. But egg protein at this level seems to be completely utilized in metabolism (Mitchell and Carman, '26 c). For a protein with a biological value of 50, assumed for the estimates in

TABLE 6

A comparison of methods of estimating the requirements of animals for digestible protein for maintenance

BODY WEIGHT OF ANIMAL		DIGESTIBLE PROTEIN REQUIRED ACCORDING TO			
		PROPOSED METHOD		THE METHOD OF ARMSBY (AND OF SHERMAN FOR MAN)	
kg.	*lb.*	*gm.*	*lb.*	*gm.*	lb.
681	1500	211	0.465	409	0.90
454	1000	157	0.35	272	0.60
227	500	94	0.208	136	0.30
91	200	48	0.106	54	0.120
68	150	39	0.086	41	0.090
45	100	29	0.064	27	0.060
2.2	5	3.15	0.007	1.36	0.003
0.2	0.44	0.540		0.120	
0.1	0.22	0.325		0.060	

table 6, 560 mg. of protein (280 × 2) would be required. The estimate given in the table for a 200 gm. animal is 540 mg., while the Armsby standard prescribes only 120 mg.

The estimates in table 6 relate only to mature animals, since the Brody equation on which they are in part based is so restricted. To estimate the requirements of protein for the maintenance of young growing animals, the Brody equations relating basal metabolism to body weight for different species (Brody, '32 b) may well be used.

CONCLUSIONS

Among the species of animals investigated (mouse, rat, guinea pig, rabbit and pig), the smaller the body size the more rapid is the adjustment to the post-absorptive condition during fasting, and the more rapid is the adjustment to the endogenous level of nitrogen excretion in the urine during specific nitrogen starvation.

Among warm blooded animals, the total endogenous nitrogen output—and hence the protein requirement for maintenance—varies more nearly with the body surface than with the body weight.

There is a close relationship between the total endogenous nitrogen excretion of warm blooded animals varying widely in size and their basal metabolism, such that 2 mg. of nitrogen are lost to the body daily for every calorie of basal heat. This relationship appears to apply also to the human species. The endogenous catabolism of an animal, regardless of species, may be estimated in this manner from its basal heat production as accurately as the latter may be estimated (under the best conditions) from its body surface or body weight. This estimate of the endogenous loss of nitrogen is the basic information necessary to compute the maintenance requirement of protein.

Although within any species a fairly constant relationship exists between the excretion of creatinine and the basal metabolism, there is no constant relationship among different species in this respect, because for different species the creatinine nitrogen characteristically accounts for different percentages of the total endogenous nitrogen. The smaller the body size in a species, the smaller the percentage of the total endogenous nitrogen represented in the creatinine output.

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RATES OF ABSORPTION AND GLYCOGENESIS FROM VARIOUS SUGARS

SIDNEY FEYDER[1] AND H. B. PIERCE

Department of Vital Economics, University of Rochester, Rochester, N. Y.

THREE FIGURES

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The question of the rate of absorption of carbohydrates from the gastro-intestinal tract is intimately related to any problem of glycogen synthesis. Cori ('25) concluded that the rate of absorption of hexoses was independent of the absolute amount and concentration of the sugar in the intestine, giving a straight line relationship between the absorption rate and time. Magee ('30) states that Auchinachie, Macleod and Magee in unpublished work had, in general, confirmed Cori's results for 'sugar.' Wishnofsky ('34), using fourteen human female diabetics, also reached the conclusion that the rate of absorption of dextrose from the human gastro-intestinal tract was the same, regardless of the absolute amount present and affirmed as a corollary to this that the rate of absorption of dextrose was constant. We feel that for the data presented only one of the three groups in his table 1 warrants such a conclusion. Pierce, Osgood and Polansky ('29) concluded that there was a falling off in the rate of absorption during the second and especially the third hour after feeding. Cori, Cori and Goltz ('29) pointed out that Pierce et al. did not feed enough sugar to enable absorption to proceed for 3 hours. Since 1929, results from several laboratories have been published showing that the rate of glucose absorption is not uniform as stated by Cori, but tends to decrease with time. Burget, Moore and Lloyd ('32) state, "The average results of our experiments on rats show a decided decrease in absorption during the second hour." These workers did not study absorption rates after 2 hours. Ravdin, Johnston and Morrison ('33), using fox hounds with jejunal loops, state, "When the amount of sugar absorbed is plotted against time, no linear relationship can be demonstrated." Burget, Moore and Lloyd ('33), studying absorption of glucose by chronic loops of colon, present data which lead us to infer that there is a more rapid absorption during the early than during the late stages of a 90-minute period, although it is difficult to tell whether the data for the periods in their table 2 are cumulative or differences for successive periods. MacKay and Bergman ('33 a, '33 b), using rats, studied the rate of absorption of glucose in relation to the previous diet and found a falling off in the absorption rate in successive hours. Trimble, Carey and Maddock ('33), utilizing Cori's technic with dogs, found no definite relationship between variations in the concentration or absolute weight of glucose ingested and the rate of absorption. As MacKay and Bergman ('33 b) have pointed out, this is apparently true under the particular conditions of their experiments. The glucose fed was increased the longer the absorption period, thus 2.12 gm. glucose per kilo were given to dogs for the 1-hour period, 3.21 gm. per kilo for the 2-hour period, and 4.26 gm. per kilo for the 3-hour period. It seems likely that the sustained average rate of absorption was due to the increasing amounts of glucose fed.

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[1] Corn Industries Research Foundation fellow.

Evidence is accumulating that the concentration of the glucose solution fed is related to the amount of glucose absorbed. Results reported by MacKay and Bergman ('33 b) and Ravdin and his associates ('33 a) show that by feeding increasing amounts of glucose or solutions of high concentration, the rate of absorption is increased.

Adam ('24) using infants, and Holtz and Schreiber ('30) using dogs, observed, by use of x-rays, the movement of sugar solutions containing either $BaSO_4$ or colloidal SiO_2 through the alimentary tract. Their data reveal the importance of the emptying time of the stomach as related to possible effects upon sugar absorption.

Ravdin and his associates ('33 b) have presented results showing how sugar solutions of varying concentration are diluted to approximately the same percentage composition prior to their absorption from the intestine when such solutions are fed as described by Cori. Thus, the sugar solution coming into contact with the absorbing surface of the intestine, having approximately the same concentration irrespective of that of the original solution fed, should not cause much change in the absorption rate where the whole alimentary tract is used.

In whichever manner absorption is studied, it is clear from the large variations obtained by Cori and associates, by MacKay and Bergman and by ourselves, that a large number of animals must be used to determine a dependable average and that the coefficients of variation must be given.

OBJECT

The object of the present investigation was twofold a) to redetermine the rate of absorption of glucose from the alimentary tract of rats; b) to compare this rate with that of other sugars requiring some digestion and to determine the relative rates of liver glycogen formation by these sugars; namely, a simple sugar, d-glucose (dextrose[2]), a double sugar, sucrose and a complex of single, double and multiple sugars, such as pure corn syrup.[3] The rate of glycogenesis was then studied in relation to the rate of absorption.

METHODS

In part a) of this study rats weighing between 120 and 270 gm. were used as experimental animals, seventeen of the 130 animals being females. The greater proportion ranged from 150 to 200 gm. in weight and were selected from a colony of inbred Wistar stock available in the laboratory. Approximately half of the animals received the stock diet mentioned by Pierce, Osgood and Polansky ('29), while the others had been fed on the standard stock diet of Purina dog chow since the time of weaning.

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[2] Supplied by the Corn Industries Research Foundation and confirmed as to purity by polariscope.

[3] Supplied by the Corn Industries Research Foundation and confirmed as to yield of total dextrose by hydrolysis.

In part b) adult male rats weighing from 150 to 300 gm. were used both for absorption and formation of glycogen in the liver. They were selected from the same colony of inbred Wistar stock. All had been fed on the standard stock diet of Purina dog chow since the time of weaning, thus reducing to a minimum differences in glycogenesis which might possibly be due to the effect of the previous diet. The sugars were compared from the standpoint of equivalent quantities administered, and a 50 per cent solution of dextrose was used as the standard. Sucrose (Pfanstiehl) was therefore fed in a 47.5 per cent solution, and corn syrup was prepared in a solution equivalent to the 50 per cent dextrose solution from a knowledge of its percentage composition, confirmed by acid hydrolysis.

TABLE 1

Composition of corn syrup

	DRY BASIS	MOIST BASIS
	per cent	*per cent*
Dextrins	56	45
Maltose	27	21.7
Dextrose	16	12.9
Ash	0.25	0.21

Total milligrams carbohydrate per gram syrup = 796

In part a) of this investigation, the rats were fasted for 24 hours and then, with but few exceptions (when 2.0 cc. were administered), fed 2.5 cc. of a 50 per cent dextrose (Merck) by stomach tube using the Cori ('25) technic. At the end of 1-, 2- and 3-hour intervals the different animals were killed by a blow on the head, the alimentary tract removed and washed, and glucose determined in the washings by the Bertrand method (Mathews, '25). All precautions cited formerly

(Pierce, Osgood and Polansky, '29) were observed. All of the data are taken from results obtained by one of us in person or by selected groups of medical students working under his immediate direction (series A, table 2). The results shown under series B were those obtained by the other author (F) working independently, while those in series C were obtained in an earlier study (Pierce, Osgood and Polansky, '29). These results are republished so that they may be compared with those of the present study, and also for the purpose of showing the effects of a 48-hour as compared with a 24-hour fast on the absorption coefficients.

In part b) the experimental animals were also fasted for a period of 24 hours, and then fed 2.5 cc. of the various carbohydrate solutions to be tested by stomach tube. At the end of 1-, 2- and 3-hour intervals, the animals were killed by a blow on the head, the livers quickly excised, the gastro-intestinal tract quickly ligated at the cardia and ileo-cecal valve, then removed, washed and the unabsorbed sugar determined.

From the data thus obtained it was possible to determine the rate of absorption of glucose in comparison with more complex sugars, as well as the relative rates at which glycogen was deposited in the tissues of the liver.

RESULTS

Absorption studies

1. Absorption of glucose. Table 2 presents a summary of the results obtained in studying the rate of absorption of glucose with three groups of rats. Series A was designed especially to supply a larger body of data than any hitherto published. To meet the criticism of our original results by Cori, Cori and Goltz ('29), larger amounts of sugar were fed to insure the presence of sufficient sugar to permit the same sustained rate of absorption as was taking place during the 1-hour absorption period. In only three of the thirty-seven rats used in series A for the 3-hour absorption period was there insufficient sugar to permit a rate of absorption as great

TABLE 2

Summary of the rate of absorption of d-glucose from the gastro-intestinal tract of rats

NUMBER ANIMALS	WEIGHT AFTER FASTING 24 HOURS	GLUCOSE			GLUCOSE ABSORBED MILLIGRAM PER HOUR PER		BASIS BODY WEIGHT		BASIS SQUARE CENTIMETER BODY SURFACE		GLUCOSE ABSORBED
		Fed	Absorbed	Unabsorbed	100 gm. body weight	Square centimeter body surface	Standard deviation	Coefficient variability	Standard deviation	Coefficient variability	
					Series A						
					1-hour						
	gm.	*mg.*	*mg.*	*mg.*				*per cent*		*per cent*	*per cent*
56	177	1268	365	903	213	1.295	70.7	33.19	0.403	31.12	29.09
					2-hour						
37	195	1160	690	470	181	1.145	47.4	26.19	0.257	22.43	59.30
					3-hour						
37	183	1149	859	290	160	0.996	44.6	27.88	0.240	24.10	74.9
					Series B						
					1-hour						
8	207	814	513	301	246	1.601	116.1	47.2	0.605	37.8	63.4
					2-hour						
9	200	743	554	189	143	0.894	23.0	16.1	0.129	14.6	76.3
					3-hour						
29	218	954	802	152	127	0.822	25.0	19.7	0.129	15.7	84.0
					Series C fasted 48 hours						
					1-hour						
9	169	731	381	350	236	1.40	59.3	25.13	0.289	20.60	52.6
					2-hour						
7	152	887	705	182	229	1.37	26.0	11.35	0.107	7.82	79.4
					3-hour						
7	157	857	788	69	168	0.99	14.6	8.69	0.078	7.85	91.9

as that noted in the 1-hour period. In all of the other animals there was more than enough glucose to permit the higher rate of absorption obtained during the first period. It will be noted from the data in table 2 under series A that an average of 290 mg. glucose remained unabsorbed after 3 hours. The condition in series B is somewhat different, for here, if absorption were to continue at the rate noted during the 1-hour period, over 1000 mg. of glucose would have had to be fed in place of the 743 mg. given. In spite of this fact, and with 189 mg. glucose remaining after 2 hours of absorption, the coefficient had dropped to 143. Certainly, there was sufficient glucose remaining to have made this value higher, as is true also for the third hour, if absorption were independent of amount present. The data in series C indicate that absorption of glucose after 48 hours' fasting is more uniform from animal to animal than after 24 hours' fasting.

Our calculation of glucose absorption as set forth in the first paper (Pierce et al., '29) has also been criticized by Cori and his associates ('29) and data presented in their table 1 show our calculations as not agreeing with their results. According to one of Cori's ('25) earlier conclusions, 50 per cent glucose is absorbed from the intestine at a constant rate. If this were true, it could be assumed that the data presented in the paper by Cori et al. ('29) for the different periods were interchangeable. Using data in their table 1, suppose we place the figures given under the column headed '0–2 hours' under the column headed '0–1 hour' and calculate the results on the basis of 1 hour of absorption instead of 2. Similarly, transpose the figures under the heading '0–3 hours' to column headed '0–2 hours' and calculate results on the basis of 2 hours' absorption instead of 3, and, finally, place the figures under the column headed '0–1 hour' under that headed by '0–3 hours' and recalculate on the basis of 3 hours. The averages given in table 1 of Cori et al. ('29) are presented in our table 3, as are the results obtained on transposing and recalculating the data in the manner just described. It can be seen that the results predicted by us are not far from those obtained by interchanging Cori's data, and the variation is not exceptional for such experiments, as may be seen from the coefficients of variability given in table 2.

Upon examining our data under series A, table 2, and calculating results on the basis of a constant percentage rate, we find an absorption of 29.1 per cent (365 mg.) of glucose during the first hour, which leaves 70.9 per cent (903 mg.) remaining in the alimentary tract. If 29.1 per cent of the remaining 70.9 per cent were absorbed during the second hour, 20.6 per cent (262 mg.) would be taken up, making the total 49.7 per cent (631 mg.) as against the value of 59.3 per cent (690 mg.) which was found. Finally, if 29.1 per cent of the remaining 50.3 per cent (637 mg.) were absorbed during the third hour, 14.6 per cent (185 mg.) would be taken up,

TABLE 3

Showing relation of Cori's results as obtained on transposing data as described in text

	0 TO 1 HOUR			0 TO 2 HOURS			0 TO 3 HOURS		
	Absorbed	Average absorbed coefficient	Absorbed	Absorbed	Average absorbed coefficient	Absorbed	Absorbed	Average absorbed coefficient	Absorbed
	mg.		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*
Data presented by Cori et al. 1929, table 1	203	203	21.7	469	235	50.5	660	220	70.9
Expected according to Pierce[1]				363	182	38.6	489	163	51.9
Transposing and recalculating as described in text	235	235	25.2	440	220	46.3	609	203	64.6
Expected according to Pierce				415	208	43.7	548	183	58.1

[1] Calculation: Quoting from Cori et al ('29). Four rats each killed 1, 2 and 3 hours after glucose feeding. The average amounts fed per 100-gm. rat were 943 mg. for the 1-hour, 931 mg. for the 2-hour and 951 mg. for the 3-hour absorption period. The average body weight of the three groups of rats was 140.5, 142.6 and 138.2 gm., respectively. Since 21.7 per cent of 943 is absorbed in the first hour, 740 mg. of sugar are left in the intestine after 1 hour; 21.7 per cent of 740 is supposed to be absorbed in the second hour, leaving 578 mg. for the third hour, and so on.

making the total absorption 64.3 per cent (815 mg.) in place of 74.9 per cent (859 mg.) which was found. These values are markedly different from those reported previously (Pierce, Osgood and Polansky, '29) and do not support our former belief that the amount of glucose absorbed at all times is a uniform percentage function of the concentration of glucose remaining in the alimentary tract. This does not lead us to conclude, however, that glucose concentration is a negligible factor, as will be shown in the following paragraph.

If we take the results in our present table 2, those of Pierce, Osgood and Polansky ('29) in their table 1, of Cori ('25) in his table 2, of Cori et al. ('29) in their table 1, and of MacKay and Bergman ('33) in their table 2, experiment 1-C, and plot the average amount of sugar available for absorption against the average sugar absorbed per 100 sq.cm. of body surface at 1, 2 and 3 hours, there is a definite slope to the curves showing a lowered absorption with decreasing amounts (fig. 1). All data plotted, with the exception of the very few of Cori et al. ('29) show a lessened absorption with a falling concentration. The agreement in different series is perhaps as good as could be expected. It is likely that the emptying time of the stomach is affected by its degree of filling and the relative filling of the intestine in turn by this emptying time. Absorption must be affected by the amount of mucosal surface exposed to the sugar.

2. Absorption of different sugars. In table 4 and figure 2 are shown the summaries of results obtained with respect to the relative absorption rates of the three carbohydrates studied in series B. The rates of absorption have been considered on the basis of absorption coefficients each of which represents the number of milligrams of sugar absorbed per 100 gm. of rat per hour. The absorption did not take place at a constant rate for the three successive hourly periods for any of the sugars. In the case of dextrose, the absorption coefficient is decreased approximately 42 per cent during the second hour of the absorption period, while during the third hour it drops to almost one-half of its initial value. The absorption rate of sucrose follows a somewhat similar course to that of dextrose, although it does not take place at as rapid a rate as does the simple hexose. In all 3 hours, although the marked decrease follows the general course evidenced by the dextrose, all values in terms of body weight are

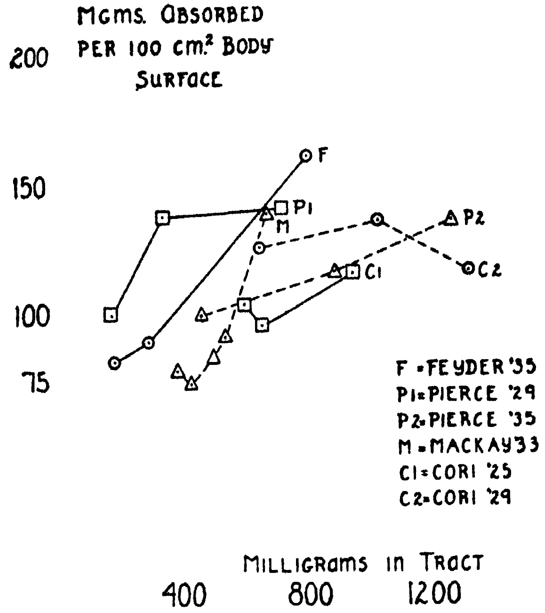

Fig. 1 Showing the average amount of sugar available for absorption plotted against the average sugar absorbed per 100 sq.cm. of body surface at 1, 2 and 3 hours. Pure Merck's dextrose was used by Pierce et al. Feyder (series B, table 2) used pure dextrose from corn. Other authors state only that 'pure glucose' was used.

TABLE 4

Summary of the rates of absorption of dextrose, sucrose and corn syrup from the gastro-intestinal tract

SUGAR	AMOUNT FED	HOURLY RATE IN TIME SHOWN AVERAGE ABSORPTION COEFFICIENTS (MG./100 GM. BODY WEIGHT)					
		Number animals	1 hour	Number animals	2 hours	Number animals	3 hours
	cc.						
50 per cent dextrose	2.5	8	246	9	143	29	127
47.5 per cent sucrose	2.5	14	212	7	119	27	108
Equivalent corn syrup	2.5	11	207	9	148	15	97

slightly below those of the simple sugar. The absorption of corn syrup from the gastro-intestinal tract is surprisingly high in comparison with the other sugars, considering that dextrins make up 56 per cent (table 1) and maltose 27 per cent of its weight on the dry basis. At the second hour it is actually slightly higher in relation to weight than either dextrose or sucrose and at the third hour it is only a little behind sucrose (fig. 2). Digestion of the dextrins evidently proceeds at times quite as rapidly as that of sucrose. Its rate of absorption is decreased approximately only 26 per cent during the second hour as compared with 42 per cent for the solutions of dextrose and sucrose, but during the third hour the rate again falls to less than one-half of its initial value. There is no support in these comparative data for Cori's idea of constant rates of absorption of different sugars.

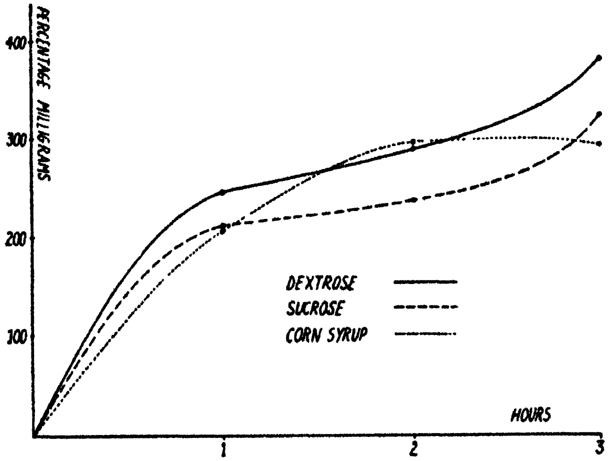

Fig. 2 Average total absorption of rats killed after 1, 2 and 3 hours.

Glycogen studies

The rate at which various sugars and carbohydrate complexes are converted into liver glycogen is a problem which has been investigated by a relatively small number of workers, most of whom have limited their investigations to a study of the simple hexoses and pentoses. It has been found, in general, that the rapidity of liver glycogenesis from various carbohydrates varies considerably with the carbohydrate administered. Cori ('26) found that when glucose, fructose or galactose were fed to animals previously fasted for 48 hours, and a group of animals killed each hour thereafter for the determination of liver glycogen, curves could be constructed for each sugar to represent the rate of glycogen formation in the liver. The curve for glucose was S-shaped, since there was relatively slow deposition of glycogen during the first 2 hours, and a marked falling off after 4 hours with a rapid deposition intermediate. Cori claims that these variations were not due to an unequal absorption of glucose from the intestinal tract of the animal, since absorption remained constant from hour to hour. The glycogen curve for fructose, Cori found to be similar to that of glucose, except that all values were slightly higher. During the absorption of galactose, he found that liver glycogen was formed one-third as rapidly as from glucose or fructose. On the basis of comparisons in which the inequalities in absorption were duly considered, Cori ('31) has concluded that of the above three sugars fructose is by far the best producer of glycogen in the liver.

EXPERIMENTAL

From all the rats used for the absorption studies in series B (table 2) the livers were taken a moment after killing and plunged into hot 30 per cent KOH for determination of glycogen, according to the method of Good, Kramer and Somogyi ('33). A control series killed after fasting 24 hours and without feeding were treated in an identical manner in order to establish a basal level of glycogen in the livers. An additional series were fed a mixture of equivalent amounts of glucose and fructose for a purpose which will be explained presently.

RESULTS

Table 5 shows the liver glycogen formation of the series of control animals after a 24-hour fasting period and of the other series similarly fasted and 1 hour, 2 hours and 3 hours after feeding. The glycogen content of the liver has been expressed on the basis of three units of reference, namely, body weight, surface area and liver weight. The average value of 1.78 mg. of liver glycogen per 100 gm. of body weight demonstrates the low basal level of liver glycogen after a fasting period of this length. Computed on the basis of surface area, this value is 1.15 mg. per 100 sq.cm. of surface area. The standard deviation computed on the basis of surface area is lower than that computed on the weight basis, but this is due to the fact that the values are also proportionately smaller. Surface area in all cases has been computed by means of Rubner's modification of the Meeh formula (Diack, '30) which is:

$$\text{Surface area in cm}^2 = 9.13 \times W^{2/3} \text{ in grams}$$

TABLE 5

Liver glycogen formation

SUGAR FED	NUMBER EXPERIMENTS	WEIGHT RANGE	TOTAL IN TIME SHOWN DIFFERENCE BETWEEN CONTROLS (24-HOUR FAST) AND EXPERIMENTALS						
			Per 100 gm. body weight	Standard deviation	Coefficient variability	Per 100 cm.² surface	Standard deviation	Coefficient variability	Per 100 gm. liver
24-hour fast									
		gm.	*mg.*	*mg.*	*per cent*	*mg.*	*mg.*	*per cent*	*mg.*
None	12	191–253	1.78	0.72	40.5	1.15	0.45	38.8	60.9
1-hour absorption period									
50 per cent dextrose	8	182–242	17.9	7.9	44.5	10.9	4.9	45.0	558.0
47.5 per cent sucrose	14	158–257	21.1	5.7	26.8	13.8	3.7	26.6	724.0
Corn syrup	11	176–296	9.8	4.3	43.6	6.5	2.9	44.7	352.0
2-hour absorption period									
50 per cent dextrose	9	162–250	40.1	8.0	20.0	25.4	5.0	19.8	1336.0
47.5 per cent sucrose	7	195–278	44.3	8.4	19.0	29.5	5.2	17.5	1542.0
Corn syrup	9	170–293	22.5	7.4	32.9	14.7	4.9	33.4	782.0
3-hour absorption period									
50 per cent dextrose	29	162–300	43.9	13.3	30.4	28.0	8.8	31.5	1557.0
47.5 per cent sucrose	27	137–384	63.2	20.0	31.8	45.8	9.9	21.6	2222.0
Corn syrup	15	228–300	34.7	8.8	25.4	24.7	6.3	25.4	1140.0
50 per cent dextrose and levulose	19	182–268	65.8	15.6	23.8	43.0	9.6	21.6	2617.0

The coefficient of variability per unit of surface area in the control animals was about 2 per cent lower than on the basis of body weight, but this difference is probably insignificant due to the extremely narrow range of weights of the animals in the series, and consequently no deduction as to whether basal liver glycogen bears a closer relationship to body weight or surface area can be drawn from the data in this table. It is evident, however, that liver glycogen is reduced to a very low level which, although not very constant from a statistical standpoint (coefficient of variability being 39 per cent), is nevertheless adequate for work where animal variation is a factor and where a difference to be expected will be great enough to show the trend of the reaction.

One hour after the administration of the three sugars studied, the data show no significant difference between the rates of glycogen formation from dextrose and sucrose (also fig. 2). The corn syrup, however, exhibits a rate of glycogen formation during the first hour which, on the average, is 50 per cent lower than that of dextrose and sucrose.

After 2 hours' absorption there is still little significant difference between the rates of glycogen formation in the liver from dextrose and from sucrose, the average difference between the two being much less than the standard deviation. The difference between these two sugars and corn syrup, as manifested during the first hour of absorption, still holds during the second hour, and the relation to the other sugars remains about the same.

After 3 hours of absorption there appears an appreciable difference in the rate of glycogenesis from dextrose and sucrose (table 5, fig. 3). The differences are clearly shown both on the basis of surface area and on the basis of body weight. Using average figures for comparison on the basis of weight, it may be stated that the rate of glycogenesis from sucrose after 3 hours of absorption is about 44 per cent greater than that of dextrose. Considering standard deviations, the difference is more significant on the basis of surface area, where the percentage increase is 60 per cent. The glycogen content of the liver of those animals to which corn syrup was administered after this interval is, on the average, approximately 20 per cent lower than that of dextrose and 40 per cent lower than that of sucrose.

Since sucrose had been demonstrated to form more liver glycogen than dextrose after a 3-hour interval, it was important to determine whether the fructose part of the sucrose molecule had been responsible for the additional liver glycogen laid down by sucrose. In a separate series of animals a 50 per cent sugar solution containing dextrose and fructose in the proportion in which they occur in the sucrose molecule was prepared and fed for a 3-hour absorption interval. The values obtained for liver glycogenesis after this period are quite similar to those obtained with sucrose. The rate of glycogen formation from this mixture of carbohydrates varies from that with sucrose by only a small fraction of the standard deviation.

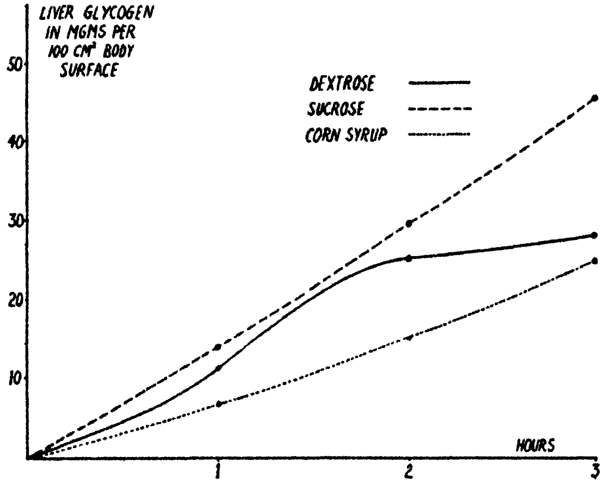

Fig. 3 Average glycogen content of livers at end of 1, 2 and 3 hours.

In the 1- and 2-hour experiments, the coefficients of variability on the basis of surface area and body weight are almost identical as would be expected, since surface area has been calculated as a power function of the weight multiplied by a constant. Also, from such a narrow range of weights of the animals used in these first two absorbing hours, a very narrow range of surface necessarily results (Murlin, '21). The series of animals used in the experiments for the 3-hour absorption period exhibit a wider range of weight variations, however, than those in other groups, the range being from 137 gm. to 384 gm., and for this reason the question of whether the glycogen content of the liver is more intimately related to body weight or to surface area can be briefly considered with respect to this group. The coefficients of variability on the two bases are approximately the same in the dextrose and corn syrup groups, but in the sucrose group the values are 32 per cent on the basis of body weight and 22 per cent on the basis of surface area. From the difference in the coefficients of variability under these conditions, one might feel inclined to deduce a closer relationship between glycogen and surface area, but the general variation and scatter of the values are much too great to allow the formulation of such a law. Liver glycogenesis obviously is related to other causal factors than the requirement for total energy.

DISCUSSION

The rate of absorption of glucose from the alimentary tract of rats has been reinvestigated, large groups of animals being used. The rate of absorption was not constant over a period of 3 hours as claimed by Cori and his associates. Rather the rate decreased with time in conformance with our former experiments and the results of various other workers cited under a review of the literature. The absorption coefficients obtained by different workers vary markedly for the 1-, 2- and 3-hour periods. For example, the coefficients obtained by Cori ('25) are lower than those obtained by Cori et al. ('29). In general, the same may be said for the results obtained by Pierce, Osgood and Polansky ('29) as compared with those presented in this paper. Cori et al. ('29) do not state whether their rats had been fasted 24 or 48 hours, but if for the former, higher coefficients would be expected as, according to Cori ('31), prolonged starvation decreases the rate of absorption. Series C of the present study does not confirm throughout the idea that 48 hours' fasting causes a lessened absorption rate (compare 2- and 3-hour periods).

With animals carefully selected with regard to age and weight, large variations were noted in the amounts of sugar absorbed and, therefore, in the absorption coefficients. One set of data may be presented to illustrate this. Three rats weighing approximately 140 gm. were fed 1100 mg. of glucose. The absorption coefficients were 294, 213 and 232. Again, three rats weighing 145, 146 and 142 gm., respectively, were fed 1495, 1495 and 950 mg. of glucose. The absorption coefficients were 228, 240 and 469. We reaffirm our former statement that with adult rats, there is no constant relationship between the body weight and absorption, even when the amounts fed are constant. Moreover, the larger amounts fed often produce less absorption than the smaller amounts, particularly in the first hour (table 2). Coefficients of variation run as high as 47 per cent. They diminish with longer periods.

Rates of absorption do not determine specifically the rates of liver glycogenesis. It has been seen, for example, that corn syrup, in spite of its complex nature, is digested and absorbed, at an average rate, equal to that of cane sugar (table 4). Indeed, at the second hour it exceeds both sucrose and dextrose slightly (fig. 3). The rate of glycogenesis, however, following the administration of this mixture on a dextrose-equivalent basis with the other sugars, is consistently much less than that of sucrose and is farthest behind dextrose at the very point where its absorption overtakes that sugar. It approximates the rate of glycogenesis for dextrose at the third hour. It must be borne in mind that absorption rates and glycogenesis rates were determined on the same animals, the liver having been excised and dropped in hot caustic alkali, just preceding the ligation and removal of the alimentary tract. The values obtained indicate that the process depends more upon chemical configuration of the carbohydrate involved, together with its effect upon the carbohydrate regulating mechanism of the organism itself, than on absolute absorption. The reverse relationship holds for sucrose, which having a slower absorption rate than dextrose at 2 and 3 hours, nevertheless formed slightly more liver glycogen than did dextrose at 2 hours and 44 per cent more at 3 hours.

Old experiments by Külz (1876) on rabbits by a method essentially the same as that which has become known in recent years as the Cori method, except that absorption was not determined, proved that fructose and sucrose are better producers of glycogen in the liver when fed in equivalent amounts than is glucose. His results were confirmed immediately by Frerichs (1876). The interval between feeding and death of the experimental animals is given as '4 to 5 hours.' The rabbits had been previously fasted for '6 full days.' Lusk (1892) showed that the formation of glycogen from fructose injected subcutaneously into rabbits took place at a more rapid rate than that from glucose, thus indicating that the difference obtained in favor of this sugar in older experiments was not dependent upon absorption rate. Cori ('26) confirmed this in rats which had been fasted 48 hours by showing that, whereas absorption of d-glucose took place twice as rapidly as fructose, the formation of glycogen from the latter was more than twice that from the former. The present experiments advance our knowledge a step further in showing that, whereas the absorption of a simple hexose takes place more rapidly than that of a double sugar, the rate of glycogen formation from the latter is more rapid. This conclusion that glycogen formation does not depend upon absorption rate is further reinforced by the feeding of a mixture of fructose and glucose. There can be no doubt that the excellence of sucrose as glycogen former is explained by the fructose fraction.

Glycogen values have also been computed throughout on the basis of liver weights, and, in general, these averages show the same tendencies as those computed on the basis of body weight and surface area. Less value in interpretation can be attached to them, however, since the variation from animal to animal is much greater on this basis. It has also been found that the relationships between liver weight and body weight as well as those between total glycogen content and liver weight are exceedingly inconsistent.

CONCLUSIONS

1. The absorption of glucose from the gastro-intestinal tract of the rat does not take place at a constant rate, but falls during a 3-hour absorption period. The same statement, in general, holds true for sucrose and corn syrup.
2. The total amount of glucose absorbed and the amount absorbed per 100 gm. body weight per hour varied within wide limits and with adult rats the variations were not dependent upon body weight. Degree of filling of the alimentary lumen is believed to play an important role.
3. Although absorption per square centimeter of surface area varied markedly, the results indicate a slightly closer relationship between absorption and body surface than between absorption and body weight.
4. There seems to be some dependence of the percentage of glucose absorbed during 1-, 2- and 3-hour periods upon the amount of glucose remaining unabsorbed in the alimentary tract, although the results in different series are not uniform.
5. The rate of glycogenesis in the liver from dextrose and sucrose is approximately the same during the first and second hours after administration of the sugars, but during the third hour the rate of glycogenesis from dextrose falls off and sucrose forms approximately 44 per cent more liver glycogen than does dextrose. This effect is due to the presence of fructose in the sucrose molecule.
6. The rate of glycogen formation from corn syrup is lower than that of dextrose and sucrose, and proceeds at a fairly constant rate during the three hourly periods of absorption.

It is our pleasure to thank Dr. John R. Murlin for the advice and guidance given us during the period of this investigation.

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FAT FORMATION FROM SUCROSE AND GLUCOSE

SIDNEY FEYDER[1]
Department of Vital Economics, University of Rochester, Rochester, N. Y.

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In the preceding paper (Feyder and Pierce, '35) it was shown that of the two carbohydrates d-glucose (dextrose) and sucrose, the latter is by far the better producer of glycogen and that this superiority is due to the fructose part of the sucrose molecule. The condition for glycogen retention in the body is, in general, identical with the condition for fat formation; that is to say, a supply of energy producing nutrients in excess of energy needs. It has often been surmised, but never proved, that glycogen is an antecedent step in the formation of fat (especially v. Wendt, '23; Hoffmann and Wertheimer, '27; Schoenen, '32). A little more light on this question would be obtained if we knew that, calorie for caloric, sucrose can produce fat more readily than glucose. Such a fact would indicate strongly, either, 1) that glycogen by reduction can be changed to fat, or, 2) that the keto hexose can be changed to fat more readily than the aldehyde hexose. In any case the outcome of such a comparison would have practical value.

Rubner (cited by Lusk, '28) found that starch, cane sugar and grape sugar are needed in the following equivalent amounts to replace 100 gm. fat in the metabolism of a fasting dog; starch 232 gm., cane sugar 234 gm., grape sugar 256 gm. But in the production of fat in domestic animals, according to the observations of Kellner ('05), starch is

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[1] The writer wishes to express appreciation to the Corn Industries Research Foundation for the fellowship which supported this work.

superior to cane sugar in the proportion of 248 to 188 (i.e., grams fat from 1000 gm. carbohydrate). In other words, while cane sugar is equal to starch as direct fuel, in laying down a reserve of fuel (fat), it is only three-fourths as effective. So far as we have been able to find from a very thorough search of the literature, neither sucrose nor fructose has been compared directly with glucose as a source of fat.

v. Wendt ('23) argues that the keto sugar should not be so effective as the aldehyde, because only 3 C atoms would be available for synthesis, whereas, according to the equation of Magnus-Levy, modified by Lusk ('15 and '28) which pictures the transformation by way of methyl glyoxal, acetaldehyde and aldol condensation, either 4 or 6 C atoms would be available. v. Wendt, however, apparently was not aware of the older evidence for the superiority of fructose as glycogen former. It is certain that sufficient evidence is not at hand on which to predict with certainty the transformation of either hexose to fat on the basis of stereochemistry. The direct evidence for the superiority of fructose as glycogen former in the rat, rabbit and dog is conclusive. It would seem that the paired-feeding method accompanied by analysis of the entire body (of rats) should establish the order of merit of the two sugars for fat formation.

EXPERIMENTAL

A group of eleven control animals taken from a colony of selected inbred Wistar stock a short interval after weaning was placed on Purina dog chow diet for a period of 7 days. At the end of this period food was withdrawn for 24 hours, the animals were killed and the bodies analyzed by a method which will be described later (p. 462). The animals to be paired and fed were selected at weaning from litter mates whose mothers had been on the same stock diet. Paired mates of the same sex and approximately the same weight (table 1) were placed in separate cages with raised wire bottoms. Special food cups so constructed as to minimize spilling and wastage were used and the weights and food consumption were recorded daily. The amount of food placed in the cups of each pair of animals daily was determined by the smaller amount eaten by one of the pair on the preceding day. Total food consumption was thus kept nearly equal day by day and was completely equalized once a week (Mitchell and Beadles, '30).

The diets fed to the two members of each pair differed only in the carbohydrate component, one of each pair receiving ordinary commercial sucrose and the other pure dextrose from corn.[2] The following represents the composition of the diets:[3]

Carbohydrate (sucrose or dextrose)	68
Casein (commercial)	18
Yeast (dried, Fleischmann's brewer's)	10
Salt mixture (Osborne and Mendel)	4
Cod liver oil, daily 3 drops	
	100

In the sucrose diet allowance was made for the fact that 95 parts sucrose produce 100 parts invert sugar upon intestinal hydrolysis, so that actually only 0.95 gm. of sucrose was ingested for each 1.0 gm. of dextrose.

In order to eliminate any possible difference in the caloric value of the two diets, which might of itself be responsible for any difference in growth or fat formation in each pair, duplicate samples of each diet were dried to constant weight and burned in the adiabatic bomb calorimeter. The dextrose diet contained 3.773 and 3.788 Cal. and the sucrose diet 3.782 and 3.779 Cal. per gram of dry food.

After the experimental animals had been on these diets for a period of 15 to 29 weeks, seven of the nine pairs were successively fasted for a period of 24 hours and the entire bodies were analyzed (p. 466).

During the course of the paired feeding on the sugar diets described above, certain symptoms appeared in various of the animals on or about the tenth week which resembled to some extent those described by Burr and Burr ('20) as evidence of the fat deficiency disease. The most important symptom seen was a marked scaling of the distal 3 to 4 cm. of the tail which in no case progressed to hemorrhage or necrosis, as is characteristic of the extreme fat deficiency. In some cases there appeared a temporary eczema on the hind feet without redness or swelling. There was no tendency to lose hair or for sores to appear on the skin. There was no evidence of hematuria or albuminuria. In pairs 1 to 4 (table 1) the scaly tail condition developed in all eight animals at the end of the tenth week, and was approximately identical both in severity and extent in the dextrose and sucrose rats. The condition improved spontaneously in pairs 1, 2 and 3, but never completely disappeared. The tail condition was most marked in the two members of pair 4, and was followed in about 4 weeks by a plateau in the growth curves of both animals. An extra supplement of 6 drops of cod liver oil daily fed to this pair equally was followed by rapid improvement of the tail condition and a resumption of growth, the sucrose animal responding slightly more rapidly.

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[2] Supplied by Corn Industries Research Foundation.

[3] Thanks are due Dr. R. G. Daggs for suggesting this diet.

In the second group of pairs carried simultaneously (viz., pairs 6, 7, 8, 9 and 10) the scaly tail condition occurred in only two-thirds of the entire group. The scaliness was of less severity than that which occurred in the first four pairs and involved only the distal 2 to 3 cm. of the tail. It appeared at the end of the seventh week in the sucrose animals and during the tenth week in the glucose animals. There was no interference with growth and the condition improved spontaneously in all animals affected, but never completely disappeared.

The diets fed contained no fat in the form of lard or butter, but no attempt had been made to produce a completely fat-free diet by extraction of the casein, yeast and cod liver oil as directed by Burr and Burr.

Results of paired feeding

In table 1 are shown in summary the results of feeding nine pairs of rats on the diets containing 68 per cent of sucrose and dextrose. In the column headed 'gain' it is apparent that in every pair the sucrose animal gained weight much faster than the dextrose rat in spite of the equality of food intake in grams and in calories. If the outcome was able to occur with equal probability in either of the two ways, that is, either the dextrose or sucrose rat might show the greater gain, then in a random selection of nine trials the frequency of its occurrence in one way would be given by the expansion of the binomial distribution $(1/2 + 1/2)^9$ (Fisher, '28). This means that its occurrence nine times in either way would result from chance, i.e., from equal effect of the two sugars, only once in 512 trials.

In the final column of table 1 are given the number of weeks each rat surpassed its mate. When weekly gains were equal, each rat was credited with 0.5 of a week. Of the 180 weekly comparisons 110.5 favored the sucrose rat and 69.5 favored the dextrose rat. The ideal outcome, if the sucrose did not affect the gain in weight more than the dextrose, would be 90 weeks favoring each sugar. The deviation from this in favor of sucrose is (110.5–90 =) 20.5. The probability of this occurrence as a mere matter of chance is found by comparing the figure 20.5 with the standard deviation of the frequency of distribution of 180 events each of which may result with equal probability. Fisher's ('28) formula for this standard deviation is—

$$\sqrt{0.5 \times 0.5 \times 180} = 6.71$$

The deviation of 20.5 from the expected outcome being 3.1 times the standard deviation of a chance distribution, it is quite clear that the former is not a matter of chance. On both methods of calculation of the odds, therefore, sucrose is shown to have been responsible for the greater increase in weight.

The analyses

It would not be proper to call this greater increase in weight 'better growth' without knowing the composition of the increase. Analyses of the entire body therefore were made,

TABLE 1

Paired feeding experiment

PAIR	SUGAR FED	SEX	INITIAL WEIGHT	FINAL WEIGHT	GAIN	TOTAL FOOD	WEEKS FED	NUMBER WEEKS IN WHICH EACH RAT SURPASSED ITS MATE
			gm.	*gm.*	*gm.*	*gm.*		
1	Dextrose	M	48	218	170	1853	22	10
	Sucrose	M	59	254	195	1853	22	12
2	Dextrose	M	66	267	201	2012	22	7.5
	Sucrose	M	62	312	250	2008	22	14.5
3	Dextrose	F	53	152	99	1990	29	11
	Sucrose	F	57	185	128	1963	29	18
4	Dextrose	F	71	212	141	1927	29	14
	Sucrose	F	66	230	164	1825	29	15
6	Dextrose	M	53	234	181	1186	16	8
	Sucrose	M	51	248	197	1193	16	8
7	Dextrose	M	66	240	174	1162	16	6.5
	Sucrose	M	63	256	193	1162	16	9.5
8	Dextrose	M	61	231	170	1180	16	5.5
	Sucrose	M	58	261	203	1180	16	10.5
9	Dextrose	F	80	172	92	998	14	3.5
	Sucrose	F	53	210	157	978	14	10.5
10	Dextrose	M	84	235	151	1235	16	3.5
	Sucrose	M	88	291	203	1232	16	12.5

1) of the control group of eleven animals selected from the same stock of weanlings as the paired rats, and 2) of all the pairs which survived in good health to the end of the feeding experiment, for protein (N), glycogen and fat. The method was as follows: The animals were killed by a blow on the

head, quickly cut into three portions, and dropped into a boiling solution of 30 per cent KOH. Solution of the tissues was complete in 15 minutes. The vessel contents were transferred to a 500 cc. volumetric flask and made up to volume after cooling to room temperature. Aliquot portions were then pipetted out for determination of nitrogen by the macro-Kjeldahl method, for determination of glycogen by the method of Good, Kramer and Somogyi ('33) and for determination of fat by the saponification method suggested by Leathes and Raper ('25) and modified by Dr. R. G. Sinclair,[4] of the Department of Biochemistry and Pharmacology. Fifty cubic centimeters of the partially saponified solution are taken and after addition of 10 cc. of absolute alcohol heating is continued for 3 hours under a reflux condenser. The KOH then is neutralized with HCl, diluted with 95 per cent alcohol to 40 per cent of the total volume and extracted with petroleum ether. After washing with water, separation in a separatory funnel and evaporation of the ether, the amount of fat is determined directly by weighing.

The composition of the control animals is given in table 2. Nitrogen is seen to be the least variable constituent, the standard deviation being only 0.083 gm. per 100 gm. body weight and the coefficient of variation only 2.9 per cent. The glycogen as milligrams per 100 gm. body weight and fat as grams per 100 gm. are about equally variable, the coefficients being 19.2 and 20.1 per cent, respectively. The method obviously is not suitable for a complete accounting of the body constituents. Preformed sugar, for example, probably is largely oxidized in the hot alkali, and ashing is out of the question. Carbohydrate in the diet, however, in so far as it had not been oxidized, might be expected to influence in a cumulative way only the three classes of organic constituents given. The protein retention and, therefore, protoplasmic growth might be affected by the sparing effect. Glycogen would be the resultant of two principal factors, aside from molecular configuration, viz., food intake and activity, but being to so large an extent a form of temporary storage the amount reported would be most affected by recent activity, i.e., since the last time of eating. Fat would most nearly represent the difference between energy supply and consumption over a long period of time, but if one form of carbohydrate were more readily converted to fat, or if a more suitable residue were left after the energy requirements were met from one of the hexoses than from the other, the difference might be expected to show in the comparison of controls with the survivors.

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[4] Thanks are due Doctor Sinclair for helpful suggestions regarding the use of this method.

TABLE 2

Body composition of control animals at beginning of diet period

RAT NO.	SEX	WEIGHT AFTER 24-HOUR FAST	TOTAL N	GRAMS N PER 100 GM. BODY WEIGHT	TOTAL GLYCOGEN	MILLIGRAMS GLYCOGEN PER 100 GM. BODY WEIGHT	TOTAL FAT	GRAMS FAT PER 100 GM. BODY WEIGHT
		gm.	*gm.*		*mg.*		*gm.*	
1	F	63			64.6	102.6	3.35	5.32
2	M	75	2.11	2.82	81.0	107.9	2.61	3.48
3	F	69	1.92	2.78	81.0	117.6	2.57	3.72
4	M	107	2.80	2.62	145.8	136.0	4.06	3.79
5	M	104	2.81	2.71	84.7	82.5	5.05	4.86
6	M	72	2.02	2.80	100.1	140.4	2.18	3.03
7	M	76	2.23	2.94	69.9	92.0	3.01	3.95
8	M	74	2.08	2.81	100.1	136.4	2.54	3.42
9	M	68	1.93	2.84	88.6	130.2	1.99	2.93
10	M	68	1.93	2.84	87.9	129.2	2.23	3.28
11	M	71	2.06	2.90	97.5	137.2	2.29	3.23
Average				2.81		110.1		3.73
Standard deviation				0.083		21.2		0.75
Coefficient variability				2.9%		19.2%		20.1%

From this point of view it is disappointing that the variation in percentage of fat contained in the controls was so large. Probably a preliminary period of equalized food intake, say, of a couple of weeks after weaning, instead of a period of ad libitum feeding, would have improved this result.

Of the nine pairs of rats fed equal quantities for as long as 14 weeks (table 1), pair 4 developed serious signs of fat deficiency, as explained on page 459, and one member of pair 9 died of pneumonia at 14 weeks. The remaining seven pairs were analyzed (table 3). Again it is seen that the protein (N per 100 gm. body weight) is the least variable constituent, and in the dextrose and sucrose rats averages almost exactly the same. The agreement in standard deviations and coefficients of variation is very satisfactory. There is only a slight increase in percentage of nitrogen over the young controls. The average difference in glycogen content is 4.6 mg. per 100 gm. body weight in favor of the dextrose animal. This is too small in comparison with the standard deviations to be significant. The average difference in fat content is 4.0 gm. per 100 gm. body weight, in favor of the sucrose group. Standard deviations and coefficients of variation on so small a number of animals are not reliably representative and therefore are not of special interest except to indicate whether the series analyzed at the end was approximately as concordant as the series of controls analyzed at the beginning. The sucrose animals were nearly as concordant, but the dextrose animals were not. The increase in percentage of fat over the control is note worthy—3.7 per cent to 13.3 and 17.3 per cent for the two groups.

On the basis of total fat content, the sucrose animal in every pair exceeded the dextrose animal by from 4 gm. (no. 8) to 31 gm. (no. 2). Without reference to the amount of difference, this result in the entire seven trials could have been obtained as a matter of chance only once in 128 trials. On the percentage basis, only six of the seven pairs showed a greater amount of fat for the sucrose animal, but even this result could occur as a matter of chance only once in sixty-eight times, since so far as known every other food factor was equalized. It, therefore, is quite clear that the greater percentage of fat produced in the sucrose rats as well as the greater gain in weight was caused by some peculiarity of the sucrose molecule. It is equally clear that of the organic constituents determined only fat was thus favored. On the basis of the superiority of fructose as glycogen former, it is prob-

TABLE 3

Body composition of rats at end of diet period

PAIR	SUGAR FED	SEX	WEIGHT AFTER 24-HOUR FAST	GAIN IN WEIGHT	TOTAL N	GRAMS N PER 100 GM. BODY WEIGHT	TOTAL GLYCOGEN	MILLIGRAMS GLYCOGEN PER 100 GM. BODY WEIGHT	TOTAL FAT	GRAMS FAT PER 100 GM. BODY WEIGHT
			gm.	*gm.*	*gm.*		*mg.*		*gm.*	
1	Dextrose	M	207	170	7.20	3.48	74.8	36.1	18.2	8.8
	Sucrose	M	239	195	6.79	2.84	99.7	41.7	34.3	14.4
2	Dextrose	M	252	201	7.85	3.12	117.6	46.8	26.4	10.5
	Sucrose	M	296	250	8.74	2.96	131.6	44.5	57.3	19.4
3	Dextrose	F	148	99	4.61	3.11	72.5	48.9	6.1	4.1
	Sucrose	F	181	128	5.59	3.09	83.8	46.3	14.4	7.9
6	Dextrose	M	225	181	5.99	2.66	155.6	69.2	47.3	21.0
	Sucrose	M	237	197	6.93	2.92	120.6	51.0	59.9	25.2
7	Dextrose	M	228	174	6.37	2.79	198.5	87.0	25.1	11.0
	Sucrose	M	244	193	8.84	3.62	181.2	74.3	35.8	14.7
8	Dextrose	M	214	170	5.43	2.54	63.8	29.8	42.1	19.6
	Sucrose	M	243	203	6.38	2.62	65.6	27.1	46.1	19.0
10	Dextrose	M	220	151	5.52	2.54	69.4	31.6	40.6	18.4
	Sucrose	M	274	203	6.79	2.48	82.5	30.1	56.8	20.8
Average	Dextrose					2.89		49.63		13.34
	Sucrose					2.94		45.04		17.34
Standard deviation	Dextrose					0.32		19.0		5.88
	Sucrose					0.34		11.8		5.15
Coefficient variability	Dextrose					11.1%		38.4%		44.0%
	Sucrose					11.5%		26.2%		28.4%

able that the greater production of fat was due to this part of the sucrose molecule, and we have one more link in the chain of evidence that glycogen formation and fat formation are intimately related.

The greater fattening effect of sucrose cannot be the result of a lesser specific dynamic effect, for the investigations of Lusk ('15), Bürger ('21) and Wierzuchowski ('31) prove that fructose has a greater effect on heat production than glucose. Furthermore, Carpenter and Fox ('30) have shown that the higher R.Q.'s following ingestion of fructose than glucose very probably are due to more rapid transformation into fat, and Carpenter and Lee ('33) have proved that no reduction of alveolar CO_2 is involved, even though more lactic acid may be produced (see Wierzuchowski for lactic acid production after intravenous injection). Finally, there was no sign of greater activity in the dextrose group, although this was not measured.

SUMMARY

1. By the paired feeding method, as improved by Mitchell and Beadles, rats were placed on diets identical in all respects, except that one member of each pair received dextrose and the other sucrose, as the chief source of energy. Bomb calorimetric determinations proved that the two diets were equal in heat value.

2. Without exception (nine pairs), the sucrose animal gained weight more rapidly than the dextrose animal. This result could not have happened by chance oftener than once in 512 trials. On the basis of the number of weeks each rat surpassed its pair mate the total result was 110.5 for the sucrose rat against 69.5 for the dextrose rat, the deviation from a chance frequency distribution being 3.1 times the standard deviation of such a distribution of 180 events.

3. Control young animals and seven pairs of the survivors after 16 to 29 weeks of feeding were analyzed for nitrogen (protein), glycogen and total fat. The results were not quite so concordant in the mature animals as in the controls, but showed clearly that the difference in the weight gains was due principally to fat.

4. It is concluded, therefore, that sucrose has a considerably greater fattening effect in the rat than dextrose.

The writer wishes to express his appreciation to Dr. John R. Murlin for his advice and assistance throughout this investigation.

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THE EFFECTS OF A DEFICIENCY OF IODINE AND VITAMIN A ON THE THYROID GLAND OF THE ALBINO RAT[1]

HELEN M. COPLAN[2] AND MYRA M. SAMPSON
Department of Zoology, Smith College, Northampton, Mass.

TWO PLATES (NINE FIGURES)

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INTRODUCTION

This investigation, dealing with the influence of dietary factors on the thyroid gland of the albino rat, was undertaken because recent direct experimental evidence challenges the generally accepted theory that endemic goiter is due to lack of iodine. The deficiency of iodine has been stated by Marine ('09), Hayden, Wenner and Rucker ('22), Tanabe ('25) and Drennan, Malcolm and Cox ('31) to cause: 1) a definite hypertrophy of the thyroid; by Knappenburg ('19), Hellwig ('31), Jackson and P'An ('32), 2) no hypertrophy; by Wegelin ('29) and Hellwig ('31), 3) atrophy; and by Marine ('09) and Thompson ('33), 4) hypertrophy followed by atrophy. Dietary defects other than iodine deficiency have also been stated to cause hypertrophy of the thyroid. McCarrison ('31) has attributed hypertrophy to vitamin A deficiency, and Hellwig ('31) has attributed it to an excess of calcium. On the other hand, Drennan, Malcolm and Cox ('31) obtained no hypertrophy in rats fed diets deficient in vitamin A or D, and Sampson and Korenchevsky ('32) obtained similar negative results in male rats fed on a diet deficient in vitamin A only. It has also been stated that iodine deficiency accompanied by some other dietary defect causes pronounced hypertrophy of the thyroid gland of rats. Sampson and Korenchevsky ('32) obtained such hypertrophy with diets deficient in vitamin A and iodine; Fischer ('33) with diets deficient in vitamin B and iodine; and Tanabe ('25), Krause and Monroe ('30), Hellwig ('31), Thompson ('32 and '33), and Levine, Remington and Von Kolnitz ('33) with iodine deficient diets containing an excess of calcium.

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[1] Contribution from the Department of Zoology, Smith College, no. 181.

[2] Trustee fellow.

Analysis of the procedure employed in the above investigations reveals that conflicting results may have been due to differences in one or more of the following factors: a) the composition of the diets which, in all except those used by Jackson and P'An and Sampson and Korenchevsky, were mixed diets of which the exact composition was unknown; b) the strain of the rats which were either Norwegian or albino; c) the initial age, as a difference in susceptibility of the thyroid gland of man to iodine deficiency at different ages is known to exist according to Marine ('09); d) the duration of the experiment, as Marine ('09) and Thompson ('33) have pointed out that an initial hypertrophy may be followed by atrophy of the thyroid; e) the sex of the rats, as it is commonly known that there is a higher incidence of goiter among women than among men in regions where goiter is endemic.

MATERIAL AND METHODS

Every attempt was made in the present investigation to eliminate all factors other than those under consideration. To ensure this the following precautions were taken: litters of albino rats were obtained from the same colony; all animals were subjected to uniform dietary procedure previous to the period of experimentation; effects of deficiencies were studied on both sexes; litter mates of the same sex and approximately the same weight were selected as controls for experimental animals; highly purified diets were used, adequate in all respects, or deficient only in the factor or factors under investigation; distilled drinking water was supplied; and experiments were run for varying lengths of time, ranging from 2 weeks to 4 months.

Litters of rats, obtained from the Department of Physiological Chemistry of Yale University, were so prepared during the period of lactation as to limit and render more uniform their reserves of vitamin A. This was done because some of the rats were to be fed a vitamin A free diet, and it was desirable to use the same preliminary dietary procedure with all animals. The resultant low reserves could be exhausted, in rats placed on vitamin A deficient diets, within a few weeks of weaning.

The age of the animals at the beginning of the experiment ranged from 22 to 28 days, with the exception of one litter which was 33 days old. The rats in each litter were weighed; those of comparable sex and weight were paired as control and experimental animals; and each one was placed in a separate cage. Seventy-seven individuals, including males and females constituting thirteen litters, were divided into the following groups designated according to their diets: adequate diet (C), iodine deficient diet (—I),[3] vitamin A deficient (—A), and doubly deficient (—A—I). Food and water were supplied ad libitum. The composition of the diets is indicated in table 1.[4]

Careful observations of the animals were made daily throughout the experiments, body weights and food consumption were recorded twice a week, indications of vitamin A deficiency were noted, and daily weights of the rats recorded when signs of deficiency first appeared. The animals on the iodine deficient diet, with their paired controls, were killed at intervals of 2 to 16 weeks. Animals on the vitamin A deficient and doubly deficient diets were killed when a loss in body weight or signs of xerophthalmia appeared, except those of litters VII, XI, XII and XIII. In these the rats were killed at a set time in order to observe the effects of the deficiencies over definite periods. Indications of vitamin A deficiency appeared at different times in different litters, ranging from 3 to 5 weeks, except in one litter in which no external sign of deficiency had appeared at the end of 2 months when the animals were killed. Professor Anderson, from whom the rats were obtained, reported similar irregularity in the exhaustion of vitamin A reserves with other litters prepared in the same manner as those used by us.

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[3] The iodine 'deficient' diet contained 16 to 17 gamma per kilogram or 16 to 17 parts per billion of iodine, according to analyses made in duplicate by Prof. Roe E. Remington. The expense of the analysis was defrayed by a special grant from Smith College.

[4] The casein used in the control diet was obtained from the Casein Company of America, that in the experimental diets from the Harris Laboratories; the salt mixture was made according to the Osborne and Mendel formula ('13), the iodine being omitted from the iodine-free salt mixture; the irradiated yeast was obtained from the Standard Brands Company; the carotene, generously provided by the companies concerned, was fed in the form of S.M.A. 'caritol' to the first two litters, as a solution of Mead Johnson crystalline carotene in maize oil to the remaining litters. Both solutions of carotene were effective in supplying adequate amounts of vitamin A. The Harris casein and the crystalline carotene were used because of their greater purity and possible freedom from iodine.

TABLE 1

The composition of diets

(C)		(—I)	
	gm.		*gm.*
Vitamin A free casein	180	Vitamin A free casein	180
Starch (corn)	530	Starch (corn)	530
'Crisco'	240	'Crisco'	240
Complete salt mixture	50	Iodine-free salt mixture	50
Yeast, irradiated	180	Yeast, irradiated	180
Carotene (2 drops of 0.3 per cent daily)		Carotene (2 drops of 0.3 per cent daily)	
(—A)		(—A—I)	
	gm.		*gm.*
Vitamin A free casein	180	Vitamin A free casein	180
Starch (corn)	530	Starch (corn)	530
'Crisco'	240	'Crisco'	240
Complete salt mixture	50	Iodine-free salt mixture	50
Yeast, irradiated	180	Yeast, irradiated	180

The animals were killed by illuminating gas. At autopsy the body weight was recorded, the amount of body fat noted but not weighed, the condition of the lungs and the vascularity of the thyroid, as indicated by color, noted carefully. All endocrine and male reproductive organs were weighed after remaining in Allen's modification of Bouin's fixative for 24 hours, according to the technic proposed by Korenchevsky ('30). The fixed material was subsequently used for histological study. Sections of the thyroid glands, 7 μ in thickness, were stained with hematoxylin and eosin, by Bensley's method ('16), and with Mallory's triple stain. Excellent results were obtained with the latter, after first treating the sections with solutions of mercuric chloride, iodine and sodium thiosulphate, as recommended by Carleton ('26). These triple stained sections, especially, were used for most of the histological study, and the other sections compared with them.

RESULTS

1. General conditions

The final body weights of the animals on the control diet, although greater than normal according to Donaldson's figures ('24) were, with one exception, below the average and in some instances below the minimum for rats of the same age from the Yale colony (Smith and Bing, '28). A comparison of final body weights demonstrates that consistent differences existed only between controls and those rats on vitamin A deficient diets in which obvious symptoms of the deficiency occurred. In the latter, loss of appetite was accompanied by fall in weight, xerophthalmia, and in a few instances by the loss of muscular coordination described by Aberle ('34). Additional evidence of vitamin A deficiency, noted at autopsy, was depletion of fat and in males degeneration of the reproductive organs. In one control, no. 41, which failed to consume the food provided, loss of weight ensued and evidence of malnutrition was noted at autopsy. In none of the rats was there any indication of respiratory or other infection. No consistent differences in vascularity of the thyroid gland, as indicated by color, were found in the animals on the deficient diets as compared with their litter mate controls.

2. Correlation of thyroid weight with body weight

The actual thyroid weight, in the majority of individuals on all diets, varied directly with the body weight, as is indicated

TABLE 2

Protocol of male rats

DIET	LITTER NUMBER	RAT NUMBER	INITIAL AGE	TIME ON DIET	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	THYROID WEIGHT	THYROID WEIGHT PER 100 GM. BODY WEIGHT
			days	*days*	*gm.*	*gm.*	*mg.*	*mg.*
(C)	VII	43	25	14	52	70	8.8	12.5
	VIII	54	27	14	41	76	11.4	15.0
	III	17	23	21	42	92	12.4	13.5
	VI	41	23	61	46	97	15.4	15.8
	VIII	75	23	20	48	105	15.6	14.8
	II	11	22	37	36	108	14.6	13.5
	IV	23	23	24	46	108	19.8	18.3
	X	62	34	14	92	123	15.4	12.5
	XI	69	25	28	42	127	24.6	19.3
	IX	56	28	24	60	140	22.2	15.8
	VI	39	23	92	42	152	18.8	12.3
	V	28	23	59	52	224	36.4	16.2
(—I)	XIII	77	23	20	46	67	12.0	17.9
	III	16	23	21	40	75	11.2	14.9
	VIII	55	27	14	44	79	14.2	17.9
	XIII	76	23	20	33	87	11.8	13.5
	X	63	34	14	86	110	16.0	14.5
	XI	67	25	28	36	115	19.6	17.0
	IV	24	23	24	42	120	13.4	11.1
	XI	68	25	28	38	124	17.8	14.3
	XI	66	25	28	30	127	18.0	14.1
	II	9	22	37	38	128	13.2	10.3
	VI	37	23	61	41	138	16.8	12.1
	VI	35	23	92	42	188	19.4	10.3
	V	29	23	59	52	219	28.6	13.0
	V	32	23	59	48	220	33.6	15.3
(—A)	III	15	23	21	44	42	5.6	13.3
	IV	22	23	24	42	64	10.8	16.8
	VII	45	25	14	44	80	12.0	15.0
	VII	48	25	14	52	82	10.0	12.2
	VII	47	25	14	52	84	8.6	10.2
	II	13	22	37	38	103	13.0	12.6
	II	12	22	37	42	130	14.4	11.0
	V	30	23	59	51	174	18.6	10.6
(—I) (—A)	III	14	23	21	44	70	10.8	15.4
	IV	21	23	24	46	72	13.4	18.6
	IX	57	28	24	67	84	14.0	16.6
	IX	59	28	24	62	95	16.2	17.0
	IX	58	28	24	66	117	14.0	11.9
	II	10	22	37	38	122	15.8	12.9
	V	31	23	59	48	148	16.4	11.1

in the protocols of the males and females as given in tables 2 and 3. Such a correlation has been shown to exist in rats by Donaldson ('24) and in rats and guinea pigs by Friedman and Loeb ('34).

TABLE 3

Protocol of female rats

DIET	LITTER NUMBER	RAT NUMBER	INITIAL AGE	TIME ON DIET	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	THYROID WEIGHT	THYROID WEIGHT PER 100 GM. BODY WEIGHT
			days	*days*	*gm.*	*gm.*	*mg.*	*mg.*
(C)	VI	40	23	47	42	63	9.4	14.9
	XII	74	26	21	27	75	10.0	13.3
	XI	72	25	14	40	80	8.8	11.0
	VIII	50	27	21	42	88	12.6	14.3
	IX	60	28	14	57	104	12.8	12.3
	I	5	28	28	55	108	12.2	11.3
	X	64	34	14	90	118	15.2	12.8
	IV	27	23	92	34	120	18.4	15.3
	V	33	23	61	46	154	23.8	15.4
	III	18	23	119	44	155	23.0	14.8
	VII	42	25	37	50	162	19.4	11.9
(—I)	VI	38	23	47	34	66	10.6	16.0
	VIII	51	27	21	40	73	13.8	18.9
	VI	36	23	47	42	84	12.2	14.5
	IV	25	23	92	42	88	14.4	16.3
	IX	61	28	14	57	94	15.4	16.3
	I	1	28	28	66	134	17.2	12.8
	IV	26	23	92	42	140	18.6	13.2
	X	65	34	14	90	146	19.4	13.3
	III	20	23	119	44	152	20.2	13.2
	III	19	23	119	46	164	22.8	13.9
(—A)	XI	70	25	14	36	46	7.2	15.6
	VIII	52	27	21	39	53	7.6	14.3
	XII	73	26	21	40	53	9.8	18.5
	XI	71	25	14	40	60	9.0	15.0
	VII	46	25	37	50	68	9.6	14.1
	I	7	28	28	58	72	11.0	15.2
	VII	49	25	37	51	76	13.6	17.9
	VII	44	25	37	44	116	17.0	14.6
	I	8	28	28	68	122	18.4	15.0
(—I) (—A)	VIII	53	27	21	37	49	7.2	14.7
	I	4	28	28	67	104	13.0	12.5
	I	3	28	28	74	111	18.6	16.7

3. *Comparative weights of thyroid glands per 100 gm. of body weight*

Table 4 includes the weights referred to below.

(*—I*) *Diet.* In male rats, an iodine deficient diet for from 14 to 21 days produced hypertrophy of the thyroid gland as compared with the glands of litter mate controls. This diet over a period of 25 to 92 days caused slight atrophy. In females hypertrophy was more pronounced than in males and persisted up to the forty-seventh day on the deficient diet. Atrophy occurred in females fed the deficient diet for periods of 47 to 119 days.

(*—A*) *Diet.* In male rats on the vitamin A deficient diet the thyroid glands were, with one exception, consistently smaller than in their litter mate controls, the contrast being most pronounced at the end of the experimental period of 59 days. In females, on the contrary, the thyroid glands were enlarged in the rats on the vitamin A deficient diet for periods of 14 to 37 days as compared with those on the control diet. The onset of symptoms of vitamin A deficiency prevented the continuance of females on this diet for longer periods, which might have resulted in atrophy of the thyroid.

(*—A—I*) *Diet.* In males, on a diet deficient in both vitamin A and in iodine, the weights of the thyroids were, with one exception, slightly greater than in their litter mate controls at the end of 24 days. In male rats fed the doubly deficient diet for 37 days, there was slight atrophy, and in those on the diet for 59 days more pronounced atrophy. In the females on the deficient diet for 21 to 28 days there was slight hypertrophy. Onset of the symptoms of vitamin A deficiency precluded the investigation of effects of more prolonged feeding with the doubly deficient diet.

4. *Effects of the diets on the histological structure of the thyroid*

(*C*) *Diet.* The histology of the thyroid of the rats on the control diet, that is, of the normal gland, is characterized by

TABLE 4

Differences in weight of the thyroid glands per 100 gm. of body weight

DIETS	MALES					FEMALES				
	Litter number	Control rat number	Experiment rat number	Time on diet	Difference in thyroid weight	Litter number	Control rat number	Experiment rat number	Time on diet	Difference in thyroid weight
				days	*mg.*				*days*	*mg.*
(-I) versus (C)	VIII	54	55	14	+ 2.9	IX	60	61	14	+ 4.0
	X	62	63	14	+ 2.0	X	64	65	14	+ 0.5
	XIII	75	76	20	— 1.3					
			77	20	+ 3.1					
	III	17	16	21	+ 1.4	VIII	50	51	21	+ 4.6
	IV	23	24	24	— 7.2					
	XI	69	66	28	— 5.2	I	5	1	28	+ 1.5
			67	28	— 2.3					
			68	28	— 5.0					
	II	11	9	37	— 3.2					
						VI	40	38	47	+ 1.1
								36	47	— 0.4
	V	28	29	59	— 3.2					
			32	59	— 0.9					
	VI	41	37	61	— 3.7	V	33	34	61	— 2.8
	VI	39	35	92	— 2.0	IV	27	26	92	— 2.1
						III	18	19	119	— 0.9
								20	119	— 1.6
(-A) versus (C)	VII	43	45	14	+ 2.5	XI	72	71	14	+ 4.0
			47	14	— 2.3			70	14	+ 4.6
			48	14	— 0.3					
	III	17	15	21	— 0.2	XII	74	73	21	+ 5.2
						VIII	50	52	21	+ 0.0
	IV	23	22	24	— 1.5					
						I	5	7	28	+ 3.9
								8	28	+ 3.7
	II	11	12	37	— 2.5	VII	42	44	37	+ 2.7
			13	37	— 0.9			46	37	+ 2.2
								49	37	+ 6.0
	V	28	30	59	— 5.6					
(-A-I) versus (C)	III	17	14	21	+ 1.9	VIII	50	53	21	+ 0.4
	IX	56	57	24	+ 0.8					
			58	24	— 3.9					
			59	24	+ 1.2					
	IV	23	21	24	+ 0.3					
						I	5	3	28	+ 5.4
								4	28	+ 1.2
	II	11	10	37	— 0.6					
	V	28	31	59	— 5.1					

marked variation, typical of the normal human gland (Marine, '09, and Reinhoff, '30); and of the duck thyroid (Severinghaus, '33). Variations occur in the structure of the thyroid in different individuals, different parts of the same gland, and different areas of the same follicle. The height of the cells lining the follicles varies from low to high cuboidal (fig. 1); mitotic figures and colloid droplets occur in some and not in other follicular and interfollicular epithelial cells; vesicles vary in size, shape and in the amount, distribution, vacuolization and staining capacity of the colloid within them. The peripheral area of the colloid in the vesicles and the intracellular colloid invariably took the basic stain in the Mallory and the Bensley technics, which indicates its lability and the fact that it is newly formed or resorbed colloid (Severinghaus, '33). The remainder of the colloid in most cases took the acid stain, but in some follicles the entire colloid mass took the basic stain. The process of transfer of colloid is evidently transcellular in view of the location of similar colloid droplets in vacuoles in the following areas: within and at the edge of the colloid mass in the vesicles, at the free border, and within the epithelial cells lining the vesicles (figs. 2 and 3). Transcellular method of resorption has been described in amphibia (Grant, '31); and in the duck, sheep, guinea pig and monkey (Severinghaus, '33). New follicles form within the follicular and interfollicular epithelium by the method described for sheep by Severinghaus ('33), and also by a constriction of large colloid masses in the follicles, and the subsequent enclosure of the two resultant masses by the reorientation of some of the epithelial cells. There is no evident relation between the occurrence of mitotic figures and new follicle formation.

(—*I*) *Diet.* In rats on the iodine deficient diet slight hyperplasia is evident at the end of 28 days and occurred in both males and females on the diet for 56 days. It is characterized by the height and particularly by the dome-shaped free edge of the epithelial cells lining the follicles (figs. 4 and 7). This hyperplasia is succeeded by atrophy in males fed this deficient diet for 84 days, and in females on the diet for 122 days. The characteristics of the atrophic thyroid gland (fig. 9) are the large size of the vesicles throughout, the flattened epithelial cells lining the vesicles, the retraction of the colloid from the free edge of the cells, staining indicating the resorption of colloid, and the greater amount of connective tissue as compared with that in the thyroid glands of litter mate controls.

(—*A*) *Diet.* The thyroids of both male and female rats on this deficient diet show two effects: 1) hyperplasia of epithelial cells similar to that observed in rats on an iodine deficient diet for a short period, and, 2) the degeneration of the epithelial cells which seems to be a specific effect of vitamin A deficiency (fig. 5).

(—*A*—*I*) *Diet.* In male and female rats on this deficient diet for 21 to 28 days, two effects appear, namely, hyperplasia and degeneration. There is no consistent difference in the colloid content of the vesicles of the deficient and the control rats nor in the number of mitoses, but there is more follicle formation. In a male rat on the doubly deficient diet for 56 days, atrophy occurred, accompanied by degeneration of the membrane and of the cytoplasm of the epithelial cells lining the vesicles (fig. 6).

When the thyroid gland of rats on the experimental diets are compared with those of litter mate controls, it becomes evident that changes in percentage weight preceded the hyperplastic, and later the atrophic, changes in the structure of the glandular epithelium. It is possible that early modifications in the weight of the thyroid are due to initial changes in its vascularity, and in the consistency of the colloid within it, which precede changes in the function and structure of the follicular epithelium.

DISCUSSION

The body weights of the rats fed the control diets are lower than those given by Smith and Bing ('28) for rats of the same age from the same colony, due probably to the fact that the preliminary dietary procedure restricted the vitamin A reserves and the resultant growth of these rats. The thyroid weights of the majority of these control rats correspond more closely to Donaldson's figures for the thyroid weights of animals of comparable age, than of those of comparable body weight. This appears to indicate that age is the factor determining thyroid weight, but that the latter is modified by body weight, as in rat no. 41. No consistent differences exist in the thyroid weights of males and females on the control diet.

The variation in the histological structure of the thyroids of rats on the normal diet is evidence of the lability of the normal gland, and favors the theory that activity of the gland is cyclic, periods of active secretion alternating with the periods of rest or of resorption of colloid, and activity of one part accompanying inactivity of another region of the same gland. The presence of mitotic figures and the prevalence of newly formed follicles indicate growth of additional thyroid epithelium in control and deficient rats. The location and the character of the colloid-containing vacuoles give evidence of the transcellular resorption of the colloid from the vesicles.

(—*I*) *Diet.* Iodine deficiency in males and females for brief periods induces hypertrophy, and over longer periods causes atrophy of the thyroid. This is indicated by the differences in weight of the thyroid glands per 100 gm. of body weight in rats on the iodine deficient diet as compared with their litter mate controls, and by the comparative histological structure of the thyroid glands of the experimental and the control rats. These observations confirm Marine's statement ('09) that iodine deficiency over brief periods may induce compensatory hypertrophy, which may with time be followed by an exhaustion atrophy. Neither hypertrophy nor atrophy occurs in rats on control diets containing iodine. The most pronounced atrophy on this iodine deficient diet occurs in rats on the diet for the longest periods, and confirms the statement of Hellwig ('31) that "the severity (of the atrophy) . . . . is in direct proportion to the length of time the feeding experiments are continued." The fact that the diet used contains iodine to the extent of 16 to 17 gamma per kilogram may account for the failure to obtain more marked hyperplasia in this investigation. The results indicate that the hypertrophy occurs only during the first few weeks after weaning, that it is transitory, and that it is followed by atrophy. Results reported by Thompson ('33) indicate that in rats the hypertrophy is more pronounced and persistent when an excess of calcium is added to an iodine-deficient diet.

(*—A*) *Diet.* Vitamin A deficiency alone led to different results in these experiments in females as compared with male rats. In the former it caused hypertrophy as indicated by comparison of thyroid weight per 100 gm. of body weight with that of litter mate controls, and as indicated by the protrusion of the free edge of epithelial cells into the cavity of the follicles. A possible explanation of the hyperplasia is that the vitamin A deficiency first alters the normal metabolism of the epithelial cells of the thyroid gland and prevents the normal absorption and utilization of iodine; and is thus a dietetic factor which causes a 'relative' deficiency of iodine (Marine, '28; and Thompson, '33). Histological examination reveals that degeneration of some of the epithelial cells occurs simultaneously with hyperplasia of others in the same follicle (fig. 5). In males the vitamin A deficiency causes atrophy, even in individuals on the diet for a very brief period. A deficiency of longer duration might produce atrophy in females. In the males the degenerating effect, so clearly evident on histological study, apparently predominates, as is indicated by the comparative weights of the thyroids per 100 gm. of body weight.

(*—A—I*) *Diet.* The effects of the iodine and the vitamin A deficient diet on the thyroid may be viewed in the light of the effects of the single deficiencies. In males for a period of 3 weeks, and in females for one of 4 weeks, the double deficiency produces slight hypertrophy as indicated by weight; but associated with it is some degeneration revealed by histological examination. In males over a period of 37 to 59 days atrophy, associated with degeneration, occurs as revealed by weight and histological appearance. It is probable that atrophy in females would also have occurred if they could have been maintained for a longer period on the doubly deficient diet.

SUMMARY

1. Iodine deficiency in the diet produces an initial hypertrophy of the thyroid gland in both males and females. This persists for a longer time in females. It is followed in both sexes by atrophy.
2. Vitamin A deficiency produces definite hypertrophy in the female, during the limited period of experimentation, but consistent atrophy in the male.
3. A deficiency of iodine and vitamin A in the diet produces an initial hypertrophy in males and females. In males atrophy follows with continued deficiency.
4. Enlargement of the thyroid gland, induced experimentally, is more pronounced and persists longer in females than in males.
5. A study of the normal rat thyroid indicates that: a) Great variability exists in the histological structure of the normal gland, which may be regarded as evidence of a cyclic secretion of the glandular epithelium; b) colloid release in the normal rat thyroid is evidently a transcellular process.

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PLATE 1

EXPLANATION OF FIGURES

Camera lucida drawings, × 1500. Colloid represented in solid black. Details added free hand from oil immersion study.

1 Portion of a single follicle of thyroid of normal rat, no. 40, female, on adequate diet 47 days. Note variability in height of epithelial cells, ranging from cuboidal to high columnar. Also size of follicle.

2 A colloid droplet outside of the vacuole in the colloid mass, passing toward the epithelium.

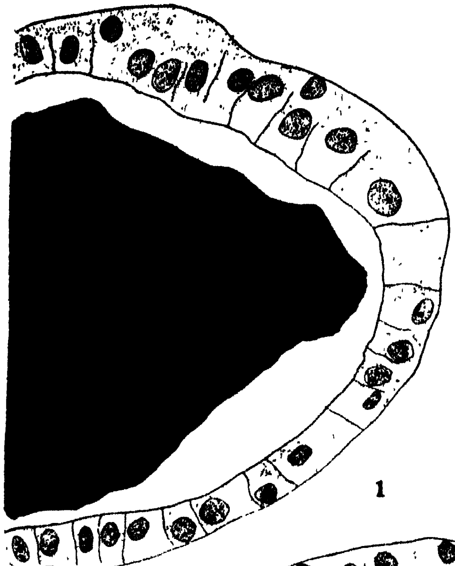
3 A colloid droplet passing through the membrane of the free edge of the epithelial cell.

4 Follicle and epithelium of adjacent follicle of rat no. 38, female, on iodine deficient diet for 47 days. Note the hyperplasia of the epithelial cells; the 'dome' shape of the individual cell.

5 Follicle of rat no. 13, on vitamin A deficient diet for 37 days. Note degenerating epithelial cells at bottom and right of follicle. Also the 'dome' shape of the free edge of cells at the left. Colloid droplets present in epithelial cells.

6 Single follicle of rat no. 21, on iodine and vitamin A free diet for 24 days. Note degeneration of epithelial cells.

PLATE 1



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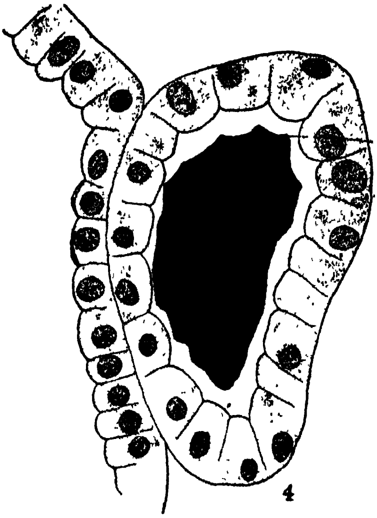

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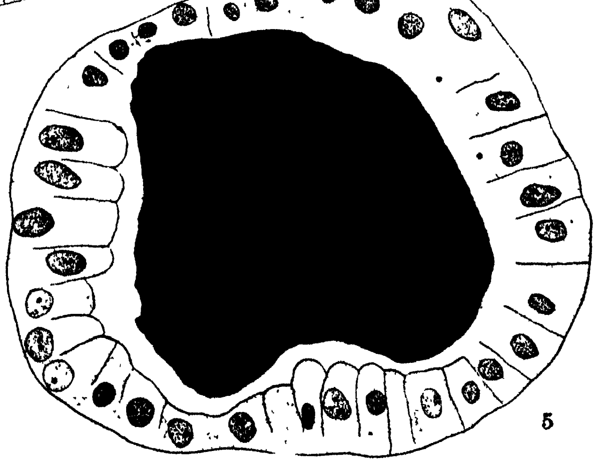

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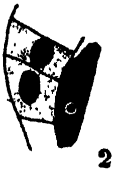

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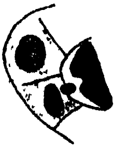

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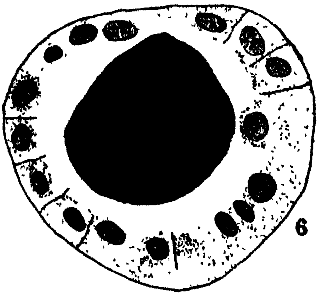

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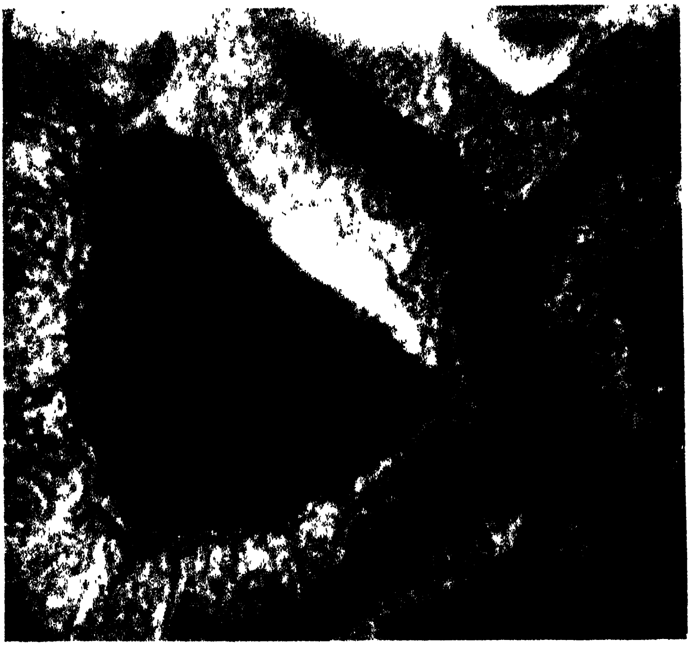
PLATE 2

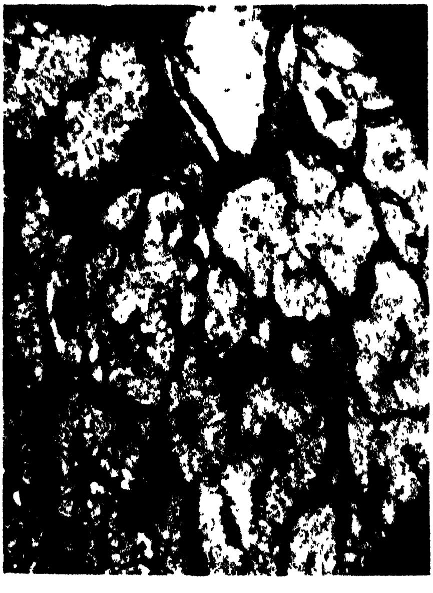
EXPLANATION OF FIGURES

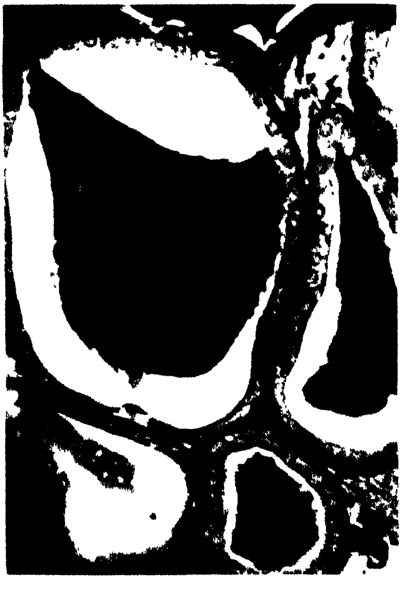
7 Follicle and epithelia of adjacent follicles of rat no. 38, female, on iodine deficient diet for 47 days. Note hyperplasia of epithelial cells. × 1400.

8 Portion of gland of control rat no. 18, female, on adequate diet for 119 days. Note many small vesicles, height of epithelial cells and small amount of colloid. Compare with figure 9 of same magnification. × 300.

9 Portion of gland of rat no. 19, female, on iodine deficient diet for 119 days. Note large size of follicles, flattened epithelial cells, and stored colloid, retracted from the epithelium. × 300.







UTILIZATION OF GELATIN, CASEIN AND ZEIN BY ADULT RATS [1]

INEZ D. MASON AND LEROY S. PALMER
Section of Animal Nutrition, Division of Agricultural Biochemistry,
University of Minnesota, St. Paul

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The relative utilization of gelatin, zein and casein was studied by McCollum ('12) in experiments on the pig. At that time relatively little was known of the mineral and vitamin requirements of animals. The present investigation was undertaken to determine the relative value of these same proteins in the nutrition of the adult rat in the light of the present knowledge of accessory needs. Since McCollum's aim was to determine the influence of a given protein in repairing waste due to endogenous catabolism, he wished to avoid feeding above the endogenous level, and took the urinary nitrogen alone as an index of the nitrogen to be fed. He regarded the fecal nitrogen as accidental in character and independent of the essential tissue metabolism, but of use in determining the thoroughness of absorption.

It is generally recognized that the biological value of a protein for maintenance is underestimated if it is fed above the endogenous level, since a portion of it would then be used for energy requirements. In the case of young growing rats, Mitchell ('23–'24 b) found that corn proteins fed at a 5 per cent level had a biological value of 72, while a 10 per cent level which permitted some growth gave a value of only 60. Whether or not the values are constant for varying levels below that required for maintenance is still a disputed question. Mitchell and Hamilton ('29) consider it logical to suppose that dietary amino acids are oxidized in the tissue ". . . . in proportion to their relative concentration in the intracellular fluids with reference to the other organic nutrients . . . . and also in proportion to their relative oxidizability as compared with glucose, fats, lipoids, etc." Hence the biological value of a protein may be expected to increase as its percentage in the diet decreases.

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[1] Part of the data reported in this paper are taken from a thesis presented by Inez D. Mason in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1933. The remainder were secured with the aid of a fellowship granted by the Graduate School of the same university. The original data are on file in the Division of Biochemistry, University of Minnesota.

Widely varying results have been given for the utilization of gelatin when fed at approximately the endogenous level. McCollum ('12), with pigs, found a utilization of 50 to 60 per cent, while Terroine, Fleuret and Stricker ('23), also with pigs, gave nitrogen balance data from which a biological value of 33 may be calculated. Mitchell ('23–'24 b), with rats, got results ranging from 0 to 44.

Casein was given a value of 70 by Thomas ('09), 71 by Mitchell ('23–'24 b) and 76 by Fixsen and Jackson ('32). Rajzmann ('33) found a biological value of 77, 78 and 81 in experiments with the rat, the pig and the rabbit, respectively.

Like gelatin, zein has given variable results. Calculations from Henriques' ('09) nitrogen balance data give a value of 30, while McCollum ('12) records a value of 80.

In this work we used Mitchell's ('23–'24 a) method for determining biological values.

EXPERIMENTAL

Preparations

Since the food intake decreases after a few days on a 'nitrogen free' diet, a study was made of palatability. The inclusion of sugar and a change of fat helped the appetite. The diet chosen contained tapioca dextrin 60 per cent, sugar 19 per cent, Osborne and Mendel's ('19) salt mixture 4 per cent, agar 2 per cent, butter fat 13 per cent, and cod liver oil 2 per cent. This was alternated with a similar diet containing slightly more lard in the place of butter fat and cod liver oil, and with slightly less sugar. These diets contained 0.22 mg. nitrogen per gram food. Allowance was made for the difference in the energy contents of the two diets in providing for the calculated energy requirements of the different animals.

In order to provide a vitamin supplement low in nitrogen, yeast was extracted as follows:

Five hundred grams of Fleischmann's whole dry yeast were extracted with 50 per cent alcohol three times, the total amount of alcohol used being 5 liters. In each extraction the mixture was heated with constant stirring, to the boiling point, then filtered through a Büchner funnel. The combined filtrates were added to 220 gm. of dextrin, evaporated, ground and made into tablets. Each tablet contained the extract from 500 mg. of yeast and weighed 300 mg. The nitrogen content of these tablets prepared from various yeast extracts was 6.2, 8.2 and 5.5 mg. per tablet, respectively.

TABLE 1

Nitrogen and water content of casein, zein and gelatin

	WATER	NITROGEN
	per cent	*per cent*
Casein	4.85	15.73
Gelatin	11.11	17.58
Zein	4.37	15.94

The casein was prepared by Palmer and Kennedy ('27), and the zein by Mason and Palmer ('34). A commercial sample of gelatin was used.

Analyses

Samples of these three proteins were analyzed for moisture by drying for 5 hours in a vacuum oven at 100° C. and for total nitrogen by the micro Kjeldahl method. The results are shown in table 1.

All analyses for total nitrogen were made with the Cavett ('31) apparatus, using the regular Kjeldahl method.

The feces were prepared for analysis by air drying, removing loose hair, and grinding. In some cases a large amount of hair was evident after the grinding. This was shaken to the top and the greater portion removed with forceps. Before weighing samples for analysis, the ground feces were again shaken and the top layer containing some hair was brushed aside so that the samples were taken from the lower layer without hair.

In determining the nitrogen distribution in the proteins a few changes were made in Cavett's ('32) modification of the Van Slyke method. The hydrolysis, distillation of hydrochloric acid and determination of ammonia nitrogen and total humin nitrogen were made in the Patton ('32) Kjeldahl-Claissen flask. Twice the quantity of material was taken throughout, the phosphotungstate separation was made by filtration and the histidine fraction obtained by calculation. The results are shown in table 2.

TABLE 2

Nitrogen distribution of casein, gelatin, zein and the alcohol soluble fecal extract after feeding zein as determined by the Van Slyke analysis. Expressed in per cent of total nitrogen

	CASEIN	GELATIN	ZEIN	ALCOHOLIC FECAL EXTRACT
	per cent	*per cent*	*per cent*	*per cent*
Ammonia N	10.30	3.86	19.68	21.39
Humin N	1.89	0.52	1.28	2.23
Arginine N	7.82	15.17	3.78	2.77
Cystine N	0.26	0.21	0.17	0.23
Histidine N	7.54	4.33	3.26	2.00
Lysine N	9.80	6.85	1.24	2.38
Total N of bases	25.42	26.56	8.45	7.38
Filtrate from bases:				
Amino N	57.71	53.06	62.40	64.24
Non-amino N	5.00	17.12	7.06	4.63
Total recovery	100.32	101.12	98.87	99.87

Experimental technic

Adult male rats weighing 350 to 500 gm. were selected at random from various laboratory strains.

The metabolism cages were made by inverting in a rack a battery of bottle tops serving as funnels. In each funnel was placed a round cage with a removable bottom. A water bottle was inserted at the side, and from the top hung a covered feed cup of the McCollum type. A copper wire screen was placed under the cage bottom in the funnel to retain the feces, while underneath the funnel was a beaker to receive the urine. The copper mesh, funnel and beaker were disinfected with an alcoholic solution of thymol to prevent decomposition of the urine, and as an added precaution against loss of ammonia 5 cc. of a 3 per cent sulfuric acid solution were placed in each beaker.

The plan of the experiment was to use a 3-day preliminary period during which the basal ration was fed from the hanging feed cups, but no collections were made. This was followed by a 2-day period in the metabolism cages. At the end of this period urine and feces were collected and analyzed. The animals were put into other cages and given in addition to the previous diet about 0.4 gm. of the protein to be used in the following period. After analyzing the urine, the amount of the desired protein which contained the same amount of nitrogen was calculated. During each of the three succeeding days each rat was given the amount of nitrogen as protein which had been excreted in urine daily by the same individual during the previous period. At the end of 3 days, collection of urine and feces was again made, and protein feeding was discontinued. After a day's interval the rats were returned to the metabolism cages for a final 2-day 'nitrogen free' feeding period, after which collections and analyses were again made. A yeast extract tablet soaked in cod liver oil was given each rat daily. The animals were kept in individual cages at all times during the experiment.

The accuracy of the collection of urine was tested by trickling a known volume of urine over each of two cage bottoms at intervals during a 3-day period. Thirty cubic centimeters of urine were used in each cage, and at the end of the period the collection was made by thoroughly washing the cage bottom, funnel and copper wire netting, using distilled water and a small brush. About 200 cc. of water were used in each case. This was made up to 250 cc., filtered, and 5 cc. duplicates taken for analysis. Analysis of the original specimen showed a loss of only about 3 per cent in collection.

Since the food intake progressively decreases on a diet very low in nitrogen, it seemed desirable to make the preliminary period as short as possible in the hope of securing an adequate energy intake throughout the experiment. In order to determine the length of time required to bring the urinary nitrogen to the endogenous level, experiments were conducted on six rats fed on a 'nitrogen free' diet. The urine was collected and analyzed daily. Only a very slight decrease was found after the third day, so a 3-day preliminary period was chosen.

It seemed desirable to feed the protein separately, since each rat was given a different amount, and it was impossible to predict from day to day just how much of the ration would be eaten. The problem was to feed the protein in such a form that it would be palatable and not easily scattered, and so that any which was thrown out could be recovered for analysis. With gelatin these conditions were met by adding 1 gm. of sucrose to each day's protein ration and then making it into a stiff gel with water. Casein and zein presented difficulties as they were easily scattered and were unpalatable in their highly purified condition. These proteins were incorporated in a sweetened agar gel. The casein was mixed with 10 cc. of a 1 per cent agar sol containing 1 gm. of sucrose and allowed to gel. Zein did not mix well with the sol, so it was beaten into the gel with a piece of copper wire. Fresh protein ration was prepared each day and at the end of 3 days all material left in the dishes as well as any which was scattered was collected and analyzed for nitrogen.

Although the basal diet contained 2 per cent of agar, the increase of this substance during the protein feeding period raised the question as to whether the increase of indigestible material in the diet altered the amount of fecal nitrogen eliminated. Mendel and Fine ('12) found that the addition of agar plus bone ash to the diet of a dog increased the nitrogen output. Mitchell ('23–'24 a) thought that the addition of indigestible matter in the form of filter paper was not of great importance in determining biological values, although he found an appreciable increase in metabolic fecal nitrogen.

In order to test the effect of this additional agar, seven rats were carried through the regular experimental periods, except that protein was not given. They were given the regular amount of extra agar as a sweetened gel during the interval and the 3-day collection period. There was no appreciable change in the amount of nitrogen eliminated per gram of food eaten.

Nitrogen balance data

Twenty nitrogen balance experiments were carried out with casein, eighteen with gelatin and twenty with zein. The relative utilization was calculated by the McCollum ('12) method in which the fecal nitrogen is not included. The results are given in table 3, the average values being 74 for casein, 23 for gelatin and 57 for zein.

The nitrogen balance data were studied to determine significant correlations and possible errors inherent in the method.

The increase in urinary nitrogen during the growth of the rat was shown by Hatai ('05). In the present experiment only adult males were used, and since the differences in weight were partly due to fat, the question arose whether there would be a larger excretion of urinary nitrogen in the heavier animals. Table 4, no. 1 shows a highly significant correlation between the weight of the animal and its endogenous urinary nitrogen even with adult rats ranging in weight from 341 to 504 gm. The tests for significance are taken from the table of Wallace and Snedecor ('31).

The following formulae were used:

$$\sigma x = \frac{\sqrt{\Sigma x^2 - (\Sigma x)\bar{x}}}{\sqrt{n-1}} \qquad r = \frac{\frac{\Sigma xy - (\Sigma x)\bar{y}}{n-1}}{\sigma x\, \sigma y} \qquad t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

During the experiments each animal lost weight. This loss was partly due to a decrease in the contents of the alimentary canal. Several rats were weighed the day after the completion of the experiment having had stock food for 24 hours. The average gain was 11 gm. The other fraction was probably chiefly loss of fat. This was only gradually regained. In

TABLE 3

Percentage retention of casein, zein and gelatin calculated by McCollum's method

RAT NO.	CASEIN	ZEIN	GELATIN
11		60.7	20.1
13			23.5
14			23.1
16	76.0		22.9
17	78.8	69.0	31.8
17		74.3	23.3
20	67.6	51.5	32.8
27	84.6		35.5
27			16.7
29	73.9	50.9	
30			17.8
33	76.3	47.3	17.1
34			17.0
35	75.0	50.0	
36	82.7		
37	70.7		40.4
38			30.3
39	76.3	49.2	22.7
41	77.7		15.1
42	74.5	58.5	14.9
45	78.2		
50	80.3		
51		72.8	
52	77.0	51.9	
52		72.9	
55	64.0		
56	72.5	59.3	
59	71.7	64.0	
60	70.0	58.9	
61	60.1	47.8	16.1
61		57.1	
63		44.1	
64		46.6	
67		59.9	
Mean	74.4	57.3	23.4
Standard deviation	5.95	9.41	7.69

TABLE 4

Summary of nitrogen balance data

FACTORS CORRELATED	n	$\bar{x}$	$\bar{y}$	σx	σy	r	t
1. x: Weight of rat y: Endogenous urinary N	30	399.8	71.4	38.96	9.25	+ 0.482	2.91
2. x: Percentage retention gelatin N y: Percentage retention zein N	7	21.6	55.9	6.59	8.46	+ 0.213	0.486
3. x: Percentage retention gelatin N y: Percentage retention casein N	10	23.6	74.3	8.41	6.74	— 0.036	0.010
4. x: Percentage retention zein N y: Percentage retention casein N	12	56.4	72.9	7.34	5.06	+ 0.255	0.834
5. x: Mg. N in yeast and basal diet y: Percentage retention zein N	20	8.5	57.3	1.78	9.41	— 0.364	1.660
6. x: Mg. N in yeast and basal diet y: Percentage retention gelatin N	18	7.9	23.4	0.81	7.69	+ 0.268	1.111
7. x: Ingested zein N as percentage endogenous urinary N y: Percentage retention zein N	20	83.4	57.3	17.54	9.41	— 0.444	2.101
8. x: Ingested gelatin N as percentage endogenous urinary N y: Percentage retention gelatin N	18	95.2	23.4	23.41	7.69	— 0.477	2.168
9. x: Ingested casein N as percentage endogenous urinary N y: Percentage retention casein N	20	105.2	74.4	9.61	5.95	— 0.318	1.424
10. x: Percentage ingested zein y: Percentage retention zein N	20	4.24	57.3	1.98	9.41	— 0.344	1.555
11. x: Percentage ingested gelatin y: Percentage retention gelatin N	18	3.98	23.4	1.86	7.69	— 0.471	2.136
12. x: Percentage ingested casein y: Percentage retention casein N	20	5.52	74.4	1.58	5.95	— 0.198	0.859
13. x: Fecal zein N as percentage ingested N y: Urinary zein N as percentage resorbed N	20	20.7	55.1	8.77	15.74	+ 0.738	4.890
14. x. Fecal corn N as percentage ingested N y: Urinary corn N as percentage resorbed N (Mitchell)	21	6.0	40.1	3.15	6.54	+ 0.608	3.334
15. x: Fecal cocoa N as percentage ingested N y: Urinary cocoa N as percentage resorbed N (Mitchell)	13	60.0	59.6	7.92	13.32	+ 0.659	2.907
16. x: Efficiency index y: Fecal zein as percentage ingested N	22	1.71	27.8	0.44	8.26	— 0.251	0.116
17. x: Efficiency index y: Urinary zein N as percentage resorbed N	22	1.71	61.7	0.44	10.65	+ 0.029	0.132
18. x: Efficiency index y: Biological value of zein	22	1.71	38.3	0.44	10.64	— 0.030	0.133
19. x: Fecal zein N as percentage ingested N (rats of known efficiency) y: Urinary zein N as percentage resorbed N	22	27.8	61.7	8.26	10.65	+ 0.102	0.462

determining the endogenous urinary nitrogen of young rats, Mitchell ('23–'24 a) introduces corrections for changes in body weight during the experiment. In order to see whether this correction is necessary with large adult rats, twelve experiments were recalculated using corrections for body weight and compared with the uncorrected values. There was no appreciable difference in the figures.

The greatest difficulty of the experiment lay in securing adequate calorie intake and many animals were discarded temporarily because they ate so little. The surface area was calculated from the modified Meeh-Rubner formula as given by Lee ('29). This for male rats is $S = 12.62 \times W^{0.60}$, where $S =$ body surface in square centimeters, and $W =$ weight in grams. Hitchcock ('27) calculates that inactive mature rats require approximately 855 calories per square meter of body surface. The amount of food required was calculated and when the intake fell far below this amount, the rat was discarded. It seemed possible that with fat mature animals a somewhat lowered food intake for a brief period might not be of great importance, since the animal's supply of fat would furnish energy. One might expect that a lowered intake of energy during the protein feeding period would give a lowered retention of nitrogen, since part of the protein would probably be used for energy requirements. On the other hand, a lowered calorie intake during the 'nitrogen free' periods might be expected to give too high a percentage retention because of giving too high a value for the endogenous nitrogen. This would mean too low a value for the exogenous fraction and in consequence a high percentage retention.

In order to determine the significance of the calorie intake, a study was made of the individual cases. Of the fifty-eight experiments there were nineteen in which the energy intake fell below the theoretical requirements in one or both of the 'nitrogen free' periods, but was adequate during the protein feeding period. This showed no appreciable effect on the percentage retention of nitrogen. Similar findings are reported by Fixsen and Jackson ('32). There were only two cases in which the intake was inadequate during the protein feeding period, but adequate during the other periods. In these two cases the values were distinctly below the average, though not so low as the lowest values in experiments in which the intake was adequate throughout. It was assumed, therefore, that no serious error was introduced into the average results due to inadequate food intake.

Since in some cases the same animals were used in experiments with more than one protein, it seemed desirable to know whether a rat which utilized one protein well would give similar results with another. Correlations were made between the percentage retention of the nitrogen of gelatin and zein, gelatin and casein, and zein and casein. Nos. 2, 3 and 4 in table 4 show no relation between the retention of the different proteins.

As the 'nitrogen free' diet and the vitamin supplement both contained a small amount of nitrogen, the question arose as to the possibility of a supplementary action between these forms of nitrogen and the protein studied. To test this the supplementary nitrogen ingested was correlated with the percentage retention of nitrogen in the case of each of the inadequate proteins. No relationship was found (table 4, nos. 5 and 6).

The constancy of the biological value of any protein for maintenance when fed in amount less than that required for nitrogen equilibrium is still a debated subject. Martin and Robison ('22) are inclined to the view that it is constant, while Mitchell ('23–'24 b) believes that the animal organism becomes more economical as less protein is supplied, so that higher values are obtained for smaller quantities ingested. He believes that the biological value is related to the percentage of proteins in the food, since a decreased calorie intake would increase the utilization of protein for energy.

In the present work each animal was given in the form of protein the amount of nitrogen which it had excreted in urine during the previous 'nitrogen free' period. Varying amounts of the offered protein were eaten, so we were able to correlate the ingested nitrogen expressed as its percentage of the endogenous urinary nitrogen with the percentage of nitrogen retention. The average of the two 'nitrogen free' periods is used in the correlations. This explains an ingestion of over 100 per cent in no. 9 (table 4, nos. 7, 8 and 9). Similar calculations were made in which the ingested protein was expressed as percentage of the food intake (table 4, nos. 10, 11 and 12). These two sets of correlations were uniformly negative, two in the first set and one in the second being significant, thus indicating a higher retention when less nitrogen is ingested.

As a check on the digestion of the proteins several fecal analyses were made for nitrogen in the experiments with gelatin and with casein. The digestion of gelatin was found to be complete and that of casein nearly so. In eight cases the biological values were calculated by the method of Mitchell and the results showed no appreciable difference from those calculated by McCollum's retention method.

On the other hand, zein was not well digested, and the digestibility varied with different animals. For this reason nitrogen determinations were made on the feces of all zein fed animals and the biological values were calculated according to Mitchell's method. These values showed an extremely wide variation, ranging from 18 to 72 with a standard deviationof 15.86. In investigating this wide range of values a correlation was run between the exogenous fecal nitrogen expressed as the percentage of the nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of the nitrogen resorbed. The purpose of this was to determine whether there was any connection between the two types of wastage of nitrogen, i.e., that lost through lack of digestion and resorption and the further loss of part of the resorbed portion through failure to utilize it in metabolism. This gave a highly significant correlation, as shown in table 4, no. 13.

In a further study of this relationship the nitrogen balance data (Mitchell, Beadles and Keith, '26) where cocoa was fed, also Mitchell's ('23–'24 b) experiment in which corn was fed at the 10 per cent level were treated in the same manner. In the case of cocoa, the correlation between the wastage of food nitrogen in the feces and its wastage in the urine was significant, and in the experiment with corn it was highly significant.

These correlations throw light on the wide range of biological values obtained when the animals vary widely in their ability to digest the protein. However, the fundamental explanation for this relationship remains to be determined.

Composition of undigested zein

Mitchell and Hamilton ('29) believe that the portion of the food protein which escapes digestion may differ markedly in its amino acid yield from the ingested food, since the different linkages in the molecule vary in resistance to digestive enzymes. According to this theory, most of the molecules may be partially broken down in the alimentary canal and the more resistant portion excreted. After feeding zein to rats, Osborne and Mendel ('14) were able to recover from 7.4 per cent to 39.5 per cent of the ingested nitrogen by extracting the feces with 80 per cent alcohol. This indicates that the zein residue is still alcohol soluble in spite of changes which may have taken place in its composition. If certain groups of little or no value in nutrition should be split off and resorbed first, most of this nitrogen would be excreted in the urine. In order to test this possibility, an alcoholic extract of the feces of zein fed rats was analyzed for nitrogen distribution.

After a preliminary period on a diet containing dextrin 54 per cent, sugar 10 per cent, salt mixture 4 per cent, agar 2 per cent, zein 15 per cent and lard 15 per cent, feces were collected from nine rats for 4 days. Samples were analyzed for total nitrogen. The metabolic nitrogen was calculated from the average of thirty previous experiments (1.91 mg. per gram food consumed). According to this calculation, 23 per cent of the food nitrogen was present in the feces. After ether extractions to remove the fat the feces were extracted with 92 per cent alcohol. The alcoholic extract contained 16.4 per cent of the ingested nitrogen, and 71 per cent of the calculated exogenous fecal nitrogen. Analysis of this extract is given in table 2. The nitrogen distribution, not being markedly different from that of the ingested zein, gave no evidence in support of this theory.

Nitrogen balance experiments with rats of known efficiency index

It seemed possible that the individual variation in ability to digest zein might be related to the inherent food utilization efficiency of the animals. To test this, twenty-two nitrogen balance experiments were carried out as before, using male rats whose efficiency index had been determined during the 6-week period after attaining a weight of 60 gm. These animals were supplied by Palmer and Kennedy ('31) and were from inbred strains having at least nine generations of sib matings and which showed great uniformity in ability to digest synthetic diets. The efficiency indices[2] ranged from 1.13 to 2.44. These indices were correlated with the exogenous fecal nitrogen expressed as the percentage of ingested nitrogen, with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen, and with the biological value. The exogenous fecal nitrogen expressed as the percentage of ingested nitrogen was also correlated with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen. None of these correlations are significant (table 4, nos. 16, 17, 18 and 19).

We suggest that the insignificant correlation in the latter case may be explained by the use of the advanced inbred strains in contrast to the former case where random sampling of rats resulted in a highly significant correlation between the fecal and urinary wastage of the food.

SUMMARY

Nitrogen balance experiments were carried out with adult male rats, using casein, gelatin and zein. The percentage retention calculated by McCollum's ('12) method averaged 74 for casein, 23 for gelatin and 57 for zein.

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[2] The efficiency index is the food required (in grams) per gram of gain per 100 gm. body weight.

Studies were made of the nitrogen balance data, first, to determine possible errors inherent in this type of experiment, and, second, to find significant correlations.

With the large adult male rats used it was found unnecessary to correct the urinary nitrogen for the loss in weight during the experiment.

A fall in calorie intake somewhat below the theoretical requirements during the 'nitrogen free' feeding period was of no apparent significance.

There was no apparent supplementary effect of the nitrogenous compounds of the yeast extract and the dextrin on the inadequate proteins used.

The percentage retention increased as less protein was ingested, even though it was never fed above the endogenous level.

When the same animals were used in experiments with more than one protein, there was no significant correlation between the individual utilization of the different proteins.

Gelatin and casein were so well digested that there was no significant difference between the percentage retention calculated by McCollum's ('12) method and the biological value calculated by Mitchell's ('23–'24 a) method. However, zein showed a poor and variable digestibility, the exogenous fecal nitrogen ranging from 4 to 36 per cent of the ingested zein. The biological values varied widely, from 18 to 72, the average being 45. This wide spread in results led to the discovery of a highly significant correlation between the exogenous fecal nitrogen expressed as the percentage of nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of nitrogen resorbed.

Further experiments with rats of known efficiency index for growth showed no relation between the inherent efficiency of the animal and the digestibility or utilization of the zein.

Analysis of undigested zein residues for nitrogen distribution furnished no explanation of the highly significant correlation between the food wastage in the feces and that in the urine.

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THE VITAMIN C REQUIREMENT OF THE GUINEA PIG

MARGARET DANN [1] AND GEORGE R. COWGILL
Department of Physiological Chemistry, Yale University, New Haven, Connecticut [2]

ONE CHART

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This study consists of a determination of the amount of vitamin C required by guinea pigs ranging from 124 to 1024 gm. in body weight. As a criterion of vitamin deficiency, the changes in microscopic structure of the roots of the incisor teeth, first described by Zilva and Wells ('17–'19) and analyzed in detail by J. A. Höjer ('24) and A. Höjer ('26), have been employed. Three reasons for collecting these data may be given. The first is the establishment of the relationship, if any, between body size and vitamin C requirement. Although it has been shown by Goettsch and Key ('28) and confirmed by Eddy ('29), that a 300 gm. guinea pig needs about 3 cc. of orange or lemon juice daily for protection against the earliest signs of scurvy, namely, the tooth changes, there seems to be no information available as to the requirements of larger or smaller animals. Would one expect a young guinea pig of 100 gm. weight and an old one of 900 gm. to require 1 cc. and 9 cc. of lemon juice, respectively, or relatively more or less? A quantitative answer to this question, in the form of a mathematical equation, might shed light on the actual factors (e.g., body mass, age, growth, or metabolic rate) possibly concerned in the body's use of vitamin C, in a manner analogous to what has already been demonstrated by Cowgill and his collaborators ('32) with respect to vitamin B.

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[1] Sterling Fellow in Physiological Chemistry, 1932–1933. Henry Strong Denison Fellow, 1933–1934.

[2] A part of the expense of this investigation was defrayed by the Research Fund of the Yale University School of Medicine.

The second reason why such data might be of value resides in the fact that they may assist in the determination of the human requirement for vitamin C in the same way that animal studies have done in relation to vitamin B (Cowgill, '34). Direct measurements of the vitamin C needs of human beings are difficult to make. Göthlin ('31) and Falk, Gedda and Göthlin ('32–'33), using the strength of skin capillaries as a criterion, have reported that man probably requires from 0.7 to 1.0 cc. of orange juice daily per kilogram of body weight, or relatively about one-ninth as much as the guinea pig. Measurement of the requirements of different sized members of the latter species might aid in understanding why the human requirement should be relatively so much smaller.

A third, though less important, purpose in our study is a possible contribution to the convenience of workers who use biological methods for the vitamin C assay of foods. The knowledge now available as to the chemical nature of this dietary factor, and the rapidly developing methods for the determination of ascorbic acid, may shortly render obsolete the use of guinea pigs in assay of food materials. Nevertheless, as long as biological assays are still employed, a formula for relating vitamin C requirement of the body size in this species might make it possible to use animals of any weight; the investigator would then not be limited to the use of guinea pigs between 250 and 350 gm.

METHODS

The diet employed was Eddy's ('29) modification of the Sherman-La Mer scorbutic ration and consisted of the following ingredients:

	Per cent
Skim milk powder, heated in a current of air at 110°C. for 24 hours	30
Butter fat	9
Sodium chloride	1
Rolled oats and whole bran, mixed in equal parts by weight	59
Cod liver oil	1

Eighty guinea pigs, studied in groups of from six to ten at a time, gave the results reported in this paper. Usually three or four animals of the same sex and approximate size were housed in a large cage. The small animals were obtained from a local dealer, while the larger ones were purchased from Prof. H. L. Ibsen, of Kansas State College of Agriculture, who has used for genetic studies strains characterized by unusually large individuals. Groups of various sizes were selected more or less at random over a period of a year, so that possible seasonal differences were avoided. For example, a group ranging from 120 to 200 gm. in body weight was studied in January, and another of similar size in May; some animals of 700 to 800 gm. were observed in April, and others in October.

After a few days, during which the guinea pigs received oats, cabbage and scorbutic food mixture, ad libitum, they were restricted to the scorbutic diet alone, supplemented with individual daily doses of fresh decitrated lemon juice, prepared as directed by the Conference on Vitamin Standards of the League of Nations (Medical Research Council, '32). The juice was given by pipette, and little difficulty was experienced in training most of the animals to take it quantitatively without spilling. Water was supplied ad libitum in drinking fountains consisting of inverted bottles provided with glass tubes. After a period of subsistence on the scorbutic diet plus limited vitamin C, in a few instances 11 days, but usually 14 days, the guinea pigs were chloroformed and the lower jaws quickly dissected out and placed in 8 to 10 per cent formalin. A superficial examination of the joints was made in about thirty of the animals, but no gross scorbutic changes were observed. The abdominal cavities were opened to ascertain whether or not any of the females were pregnant. It was found that the larger animals (700 gm. or over) always contained a relatively greater deposit of fat around the kidneys and genital tracts. In nineteen cases, the adrenals were removed for silver nitrate staining by the technic described by Moore and Ray ('32).

The weights given are those of the animals just before the period of subsistence on the scorbutic diet. A certain degree of inaccuracy cannot be avoided here, inasmuch as the alimentary tracts of some animals may have contained more material than others; also, most of the smaller guinea pigs were growing rapidly during the 2 weeks' period, while some of the largest ones had a tendency to lose weight.

Another possible source of error, of which we did not become aware until the experiments were well under way, is the variation which some observers have detected in the vitamin C content of lemon juice. Bennett and Tarbert ('33), working in Sicily, using the dichlorophenolindophenol titration, found orange juice higher and more consistent than lemon juice in vitamin C content. This was confirmed by Harris and Ray ('33 b) in Great Britain, who found that orange juice consistently contained 0.6 mg. of ascorbic acid per cubic centimeter; the 'best' lemon juice equalled this, while inferior lemons yielded only 0.4 mg. of ascorbic acid per cubic centimeter. In our experiments, begun before the above cited papers were published, we decided on the use of decitrated lemon juice because it was the international standard. In the light of recent knowledge, ascorbic acid, rather than either orange or lemon juice, would be the ideal material to use in an investigation of this sort.

The sections of the incisor roots were prepared by the Department of Pathology, who have kindly furnished us with a description of the method which they developed. In brief, it consisted of a double embedding, first in celloidin, followed by decalcification with 5 per cent nitric acid, and then in paraffin. Sections of 7 μ were cut under 60 per cent alcohol on a celloidin microtome, and ordinary hematoxylin-eosin staining was employed. This method differs from the original one of Höjer ('26) and from the modifications described by Goettsch and Key ('28) and by Key and Elphick ('31) as well as that used in Prof. W. H. Eddy's laboratory,[3] but the slides revealed the changes as described by these investigators. Diagnosis was made on the 0 to 4 scale of Key and Elphick, in which 4 indicates complete protection. The slides were evaluated without reference to the other data on the animals, and in all cases where there was any room for doubt, the decision represents the judgment of both authors.

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[3] Personal communication.

RESULTS

A plot of all the data is presented in chart 1. The abscissae and ordinates represent vitamin C dosage and body weight, respectively; different results are indicated by appropriate symbols. The points at which complete protection from scurvy occurred—value 4 on the scale used—are represented by solid black circles, and are of greatest significance. The light circles indicate degree of protection 3, and the triangles 2 or less. For the purpose of the present investigation the distribution of the black circles, representative of complete protection, is of particular interest. The dotted line has been drawn to show how strongly these points indicate a definitely linear relationship between the weight of the animal and the dose of lemon juice necessary to protect it from scurvy as shown by examination of the teeth.

Tabulation of the limited number of cases which includes the heaviest animal protected by each dose (table 1) reveals that, with but two exceptions, the protective dose ranges from 0.72 to 1.22 cc. of lemon juice per 100 gm. of body weight; the average value for nine different dosages, varying from 1.5 to 9 cc., proves to be 0.97 cc. (approximately 1 cc.) per 100 gm. of body weight. This result is further supported by consideration of the number of borderline cases in which the dose was evidently just below the protective amount, resulting in a microscopic section of the incisor root which justified a diagnosis of '3.' The reason why we consider it valid to ignore the two exceptional cases is that, as may be seen from chart 1, in each instance where an unusually big guinea pig was protected by a given dose (1 cc. and 3.5 cc.) at least two smaller animals failed to be protected by this amount. The reason for the exceptions is not apparent.

In only two instances were the animals found to be pregnant. These cases are too few to suggest any conclusion, except that they present no evidence of an appreciable effect of pregnancy on the vitamin C requirement.

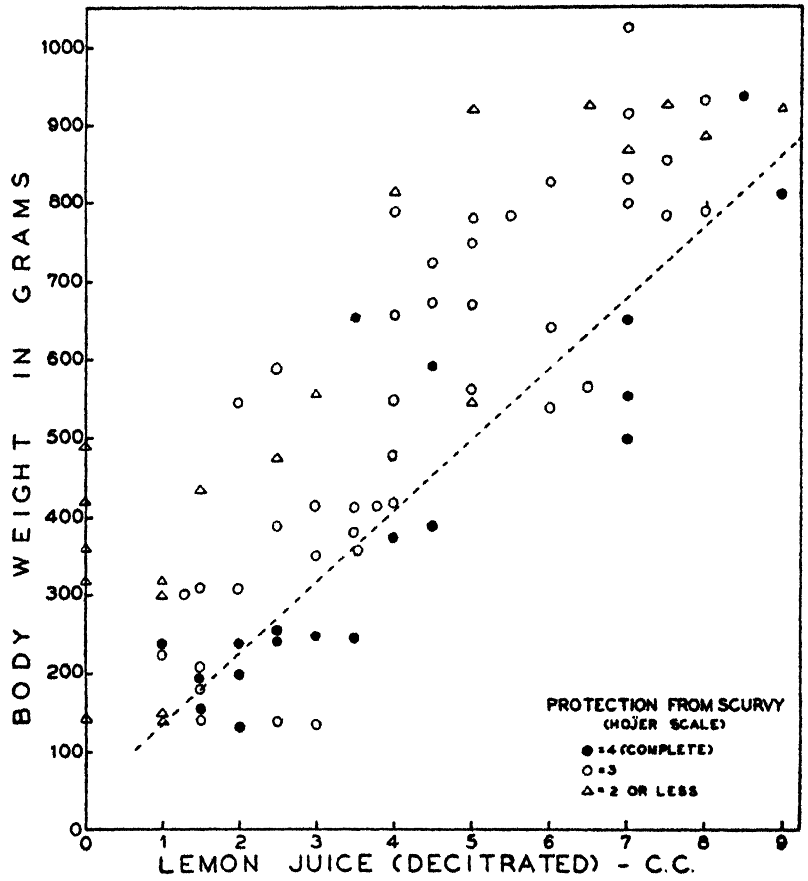

Chart 1 This plot of the body weights of guinea pigs against cubic centimeters per day of decitrated lemon juice (1932 International standard for vitamin C) indicates the degree of protection afforded against pathological changes in the odontoblastic cell layers of the roots of the lower incisor teeth. The black circles represent complete protection or a value of 4 on a scale of 1 to 4; the open circles indicate almost complete protection or a scale value of 3; in the cases represented by the triangles the protection afforded is almost nil, the scale values being 2 or less. It will be noted that the amount of vitamin C required to secure complete protection—black circles—is practically in linear relation to the body weight of the animals, and very nearly equal to 1 cc. of the decitrated lemon juice per 100 gm. of body weight.

With respect to the effect of age on the vitamin C requirement, the data suggest that the need of the youngest animals in proportion to body weight may be slightly less than that of the older groups; the observed difference is so small, however, that it may not be significant. At any rate, it is quite definitely apparent that there is no evidence of any added requirement because of the more rapid growth of the guinea pigs weighing less than 250 gm.

TABLE 1

Largest guinea pig protected by any given dose

DOSE	WEIGHT	DOSE
cc.	*gm.*	*cc. per 100 gm.*
1.0	227[1]	
1.5	209	0.72
2.0	237	0.84
2.5	252	0.99
3.0	245	1.22
3.5	652[1]	
4.0	373	1.07
4.5	589	0.76
7.0	648	1.08
8.5	935	0.91
9.0	805	1.11

[1] These cases are anomalous because at least two smaller pigs were not protected by the same dose.

In the animals studied in the winter of 1933–1934, the adrenals were stained with 0.4 per cent silver nitrate and evaluated on a purely qualitative basis. This was done in four groups of guinea pigs, containing from four to nine individuals in each group; as each group was sacrificed, the adrenals of all the members were stained for exactly 15 minutes each, and arranged in the order of intensity of the dark color. Considerable variation was observed. After the tooth slides were ready, this order was compared with the diagnosis and also with the dosage. It happened that in each group there was only one animal which was rated '4' on the Key and Elphick scale, and in every instance this turned out to be the one whose adrenals had stained most deeply, and also to be the one which had received the largest dose of lemon juice in proportion to its body weight. This result is in accord with what might have been expected from the work of Moore and Ray ('32). However, in the cases of the animals not completely protected, little or no correlation between the intensity of staining and the degree of protection could be discovered. This observation substantiates the later work from the same laboratory (Harris and Ray, '33 a) as well as the earlier statement of Gough and Zilva ('33), that the failure of adrenal tissue to stain deeply with silver nitrate cannot be relied on as a test for the absence of vitamin C.

DISCUSSION

The data here reported seem to warrant the statement that the amount of vitamin C needed to protect a guinea pig from changes in the microscopic structure of the incisor roots is proportional to its body weight over a range of 120 to 1000 gm. To assert further that the vitamin C requirement of the whole body is proportional to body weight in this species obviously involves the assumption that the tooth changes furnish a reliable index of early vitamin C deficiency of the entire organism. The validity of this assumption seems to be unquestioned by those who have used this method. Nevertheless, it might be argued that the odontoblasts, the dentine-forming cells which respond most conspicuously to diminished supply of vitamin C, might have a requirement for this dietary factor different from that of cells elsewhere in the body, because they are concerned in the growth of a structure which progresses throughout life. Dalldorf and Zall ('30) have shown that the normal rate of growth of a guinea pig's incisors is continuous, and is only slightly less in animals of 110 gm. body weight than in those weighing 600 gm. However, since the rate is practically independent of body size, one would expect that if the odontoblasts have any peculiar vitamin C requirement, this would also be independent of body size. The very fact that we have shown the requirement for normal tooth structure to vary directly with body size seems conclusive evidence that deviation from the normal in this respect is indeed an index of the entire body's deficiency.

If, then, we are justified in asserting that the direct ratio between dosage and body size is valid, the relationship can be expressed by the simple formula:

$$\text{VITAMIN}_{\text{per day}} = \text{K.\,WEIGHT}$$

Because of the units used to express the two variables in the present work, namely, cubic centimeters of decitrated lemon juice and 100 gm. body weight, the value of K happens to be unity. It is obvious that the value would be much less if ascorbic acid were used for the assay instead of lemon juice. Harris and Ray ('33 b) state that, with respect to ascorbic acid, "the minimum dose for full tooth protection (under the conditions of the test) is 2 mg." In discussing the variable potency of lemon juice, these authors also report that 1 cc. of 'good' lemon juice is equivalent to 0.6 to 0.7 mg. of ascorbic acid. Using these data, it appears from our findings that the guinea pig requires about 0.6 to 0.7 mg. of ascorbic acid per 100 gm. body weight per day. For animals of the size commonly used in vitamin C assay, namely, 250 to 350 gm. weight, the daily minimum of ascorbic acid would be from 1.5 to 2.1 mg. The higher figure, taken as the safer minimum to use, agrees very well with the minimum as stated by Harris and Ray, namely, 2 mg. Our results expressed in the 1932 International Vitamin C units based on decitrated lemon juice, indicate that the guinea pig requires 10 International units per 100 gm. body weight per day; based on the 1934 International Vitamin C unit of 0.05 mg. ascorbic acid,[4] the requirement would be 12 to 14 units per 100 gm. body weight.

The relationship between the requirement for vitamin C and body weight in the one species studied is much simpler than that found by Cowgill and co-workers ('32) for vitamin B, expressed by the formula:

$$\text{VITAMIN}_{\text{per day}} = \text{K}_{\text{s}}\text{.\,WEIGHT}^{\frac{5}{3}}$$

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[4] Personal communication from Dr. E. M. Nelson, of the Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

in which the value of K_s is peculiar to the species. This expression was found valid for pigeons, rats and dogs. The term $\text{WEIGHT}^{\frac{5}{3}}$ can be considered as equivalent to $\text{WEIGHT} \times \text{WEIGHT}^{\frac{2}{3}}$, and in interpreting this with respect to the vitamin B requirement of several different species, Cowgill concluded that "the vitamin requirement per unit of mass, i.e., weight, is proportional to the metabolism of that mass, that is, the two-thirds power of the weight."

The difference between the two formulas cited above merits some discussion. In the case of the expression pertaining to vitamin B, where the five-thirds power is the function of the body weight of interest, the formula was interpreted to indicate that not only mass (three-thirds power of the weight), but rate of metabolism (two-thirds power of the weight) as well, is an important determinant of the vitamin requirement. The absence of this latter factor from the vitamin C formula suggests that rate of metabolism does not play a role in determining the need for ascorbic acid. Against this view one may mention the fact that vitamin C is not merely an inactive component of certain tissues, but must be involved in metabolic transformations resulting in its destruction or inactivation because it is necessary to supply the vitamin frequently in order to obviate the onset of deficiency symptoms. If one wishes to adopt the suggestion of Wolbach and Howe ('26) and think of each vitamin as playing some important role in the functioning of a particular tissue, one would doubtless relate vitamin C to connective tissue, which has a relatively low rate of energy exchange in comparison with other tissues of the body; and on this latter fact one might base an explanation of the finding that rate of metabolism seems to play no role in determining the vitamin C requirement. In all probability the rate of metabolism exerts some influence on the need for vitamin C, but one which is extremely small in comparison with its effect on the vitamin B requirement, and therefore not appearing as a significant variable in the derived formula. It would be of interest to determine the effect of administration of thyroid tissue or of thyroxin on the requirement for vitamin C. The results of such studies should contribute much in relation to this question.

Our findings with reference to the guinea pig seem to be at variance with those of Falk, Gedda and Göthlin ('32–'33), who report that the child needs two to three times as much vitamin C in proportion to body weight as the adult human being. In this connection it is pertinent to point out, first, that their criterion of vitamin C deficiency consists in testing the strength of skin capillaries, and is therefore very different from ours, which is based on a study of the condition of the roots of the incisor teeth; and, second, that the two sets of observations were made on different species. It is possible that a species difference exists, especially in view of the fact that growth in the human being, up to the age of puberty, is enormously slower than in the guinea pig and other animals. This difference is beautifully illustrated by the growth charts of Brody ('27). We might also point out that the conditions of our experiments were not framed to deal critically with the growth factor. Until experiments on guinea pigs especially aimed at elucidation of the effect of growth per se on the vitamin C requirement have been performed, we do not feel justified in drawing conclusions with respect to this point.

We wish to thank Dr. Marianne Goettsch for suggestions relating to the preparation of the microscopic sections of the incisor roots and for lending us a set of standard slides. We also wish to express our appreciation of personal communications received in response to our inquiries from Dr. Kathleen M. Key, and from Dr. Celia Gurin, of Dr. W. H. Eddy's laboratory, which were helpful in formulating our plan of procedure in this investigation.

SUMMARY AND CONCLUSIONS

The vitamin C requirement was determined for eighty guinea pigs weighing between 120 and 1040 gm. Each animal, while subsisting for 2 weeks on a scorbutic diet, was given a daily dose of decitrated lemon juice varying, for different

individuals, from 0 to 2.2 cc. per 100 gm. of body weight, but mainly in the range of 0.5 to 1.5 cc. per 100 gm. The animals were chloroformed and the degree of protection from scurvy, as indicated by the microscopic structure of the roots of the incisor teeth, was evaluated by Key and Elphick's scale, adapted from the Höjer method.

The results indicate that the vitamin C requirement of the guinea pig is directly proportional to the body weight, and is almost exactly 1 cc. of lemon juice per 100 gm. This confirms the observations of Goettsch and Key, Eddy and other investigators, for guinea pigs of about 300 gm. of body weight, and applies to a much wider range of body size.

There is no evidence from these data that the young, rapidly growing guinea pig requires a proportionately greater amount of this dietary factor than the adult. This conclusion differs from that of Göthlin and his co-workers with respect to the human requirement, and may be due to a species difference.

Silver nitrate staining of the adrenals was found to be of only slight value as a criterion of the degree of protection from scurvy, as compared to the more accurate technic based on the changes in the roots of the incisors.

The role of the metabolic rate, which in the case of vitamin B has been found to be of equal importance to body weight as a determinant of the requirement of various species for the vitamin, appears insignificantly small so far as vitamin C is concerned.

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THE EFFECTS OF LACTOSE ON GROWTH AND LONGEVITY

E. O. WHITTIER, C. A. CARY AND N. R. ELLIS

Research Laboratories, Bureau of Dairy Industry, and Animal Husbandry Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington

FIVE FIGURES

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INTRODUCTION

In spite of the tendency in times past for pediatricians to emphasize the advantages of the high degree of assimilability of certain carbohydrates, such as sucrose, maltose and glucose, and to discourage the use of lactose in infant feeding, it has, more recently, been generally recognized that lactose has several uniquely characteristic properties that, at least for certain nutritional purposes, are of definite value. The authority on which lactose was to some extent discarded in favor of other sugars was derived from very meager clinical evidence and from unjustified interpretations of results of evidently sound experimentation. Even the pertinence of the fact that the solids of human milk contain 50 per cent lactose seems to have been frequently disregarded in considerations of synthetic diets for infants. This whole question has been discussed by Jarvis ('30), who was one of several to reinvestigate the effects of lactose in infant feeding.

Jarvis ('30) concluded from work with infants and from related experiments with rats that "age-weight for age-weight, the lactose-fed infant possesses more living tissue than does the infant fed on vegetable sugar." Barenberg and Abramson ('30) carried out similar work, using as much as

15 per cent of lactose in the ration, and found that infants receiving milk sugar responded with a better rate of growth than did the control group. Ariyama and Takahasi ('29) found that, in spite of the comparatively small coefficient of absorption of lactose observed by them, it had more effect on the growth of rats than sugars having greater absorption coefficients, especially in the early part of the feeding period. Mitchell ('27), feeding rats on rations containing 60 per cent of each of five common carbohydrates, respectively, found growth to be the poorest on lactose on account of lower digestibility and resulting diarrhea. Bergeim ('26) and several other investigators (Gross, '27; Robinson et al., '29), more recently, have shown that lactose feeding causes considerable increases in the amounts of calcium and phosphorus absorbed.

With the purpose of verifying some of these results on the effect of lactose feeding on the growth of young animals and of determining whether lactose has any significant effect on the body weights of adult animals and on longevity, feeding experiments with rats and pigs were carried out at the Beltsville, Md., laboratories of these bureaus.[1] Comparisons were made of body composition of animals fed lactose with that of animals fed other carbohydrates.

DESCRIPTION OF EXPERIMENTS

Three groups of rats were used in this work. Group I consisted of fourteen pairs used for the comparison of the effects of lactose and sucrose on growth and longevity and nine pairs used for the comparison of the effects of lactose and dextrin. Each pair consisted of litter mates of the same sex, but different ages. Up to the time of being placed on the experiment, the rats had received the stock ration of the breeding colony. From then on they were fed ad libitum either the lactose ration, the sucrose ration, or the dextrin ration, as listed in table 1. The lactose used was a commercial product, containing, presumably, all the vitamins usually present in this grade of lactose. Figure 1 shows the average changes in weight with age of each sex group on lactose and on sucrose. Figure 2 shows similar data up to 180 days of age for the rats paired on lactose and dextrin.

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[1] S. S. Buckley (since deceased) and Paul E. Howe, Bureau of Animal Industry, planned the details of the work with pigs. Charles Hufnagel, Bureau of Dairy Industry, was in charge of feeding the rats. P. N. Peter (since deceased), S. P. Gould and L. A. Shinn, Bureau of Dairy Industry, did the analyses on the rats.

Several pairs of rats on the sucrose-lactose comparison were killed at 132 to 159 days of age and were analyzed. Results of these analyses are given in table 2. Nine pairs were continued on the sucrose and lactose rations to obtain data on the relative effects of these sugars on the span of life. Longevity data are given in table 3. In only a few cases was it possible to determine by post-mortem examination a definite pathological cause of death. Pneumonic indications, lung abscess, enlarged spleen, tumor, and paralysis were observed, but none of these was consistently the immediate cause of death.

TABLE 1

Composition of the rations fed to rats in the different groups

RATION	COMPOSITION									FED TO RATS IN GROUPS
	Casein[1]	Salt mixture[2]	Cod-liver oil	Agar	Yeast[3]	Lard	Dextrin	Lactose[1]	Sucrose[1]	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
I (lactose)	18	4	2.5	2	5	5	33.5	30.0	00.0	I and II
II (sucrose)	18	4	2.5	2	5	5	33.5	00.0	30.0	I and II
III (dextrin)	18	4	2.5	2	5	5	63.5	00.0	00.0	I
IV (45% lactose)	18	4	2.5	2	5	5	18.5	45.0	00.0	III
V (45% sucrose)	18	4	2.5	2	5	5	18.5	00.0	45.0	III
VI (63.5% lactose)	18	4	2.5	2	5	5	00.0	63.5	00.0	III
VII (63.5% sucrose)	18	4	2.5	2	5	5	00.0	00.0	63.5	III

[1] Commercial.

[2] Osborne and Mendel.

[3] Northwestern Yeast Company (Chicago), 'Yeast Foam Tablets Powder.'

Group II consisted of ten pairs, each pair being litter mates of the same age. This group was started on the experiment

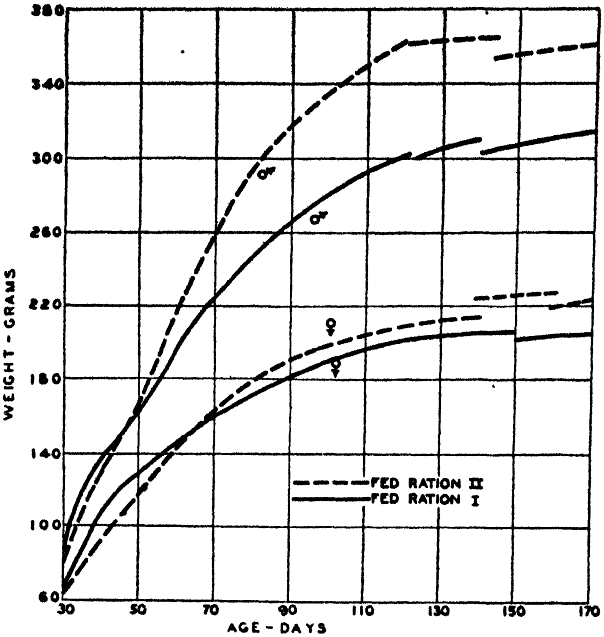

Fig. 1 Growth of rats fed ad libitum on rations I (lactose) and II (sucrose). Breaks in the curves occur, because some of the rats were killed for analysis during the feeding period.

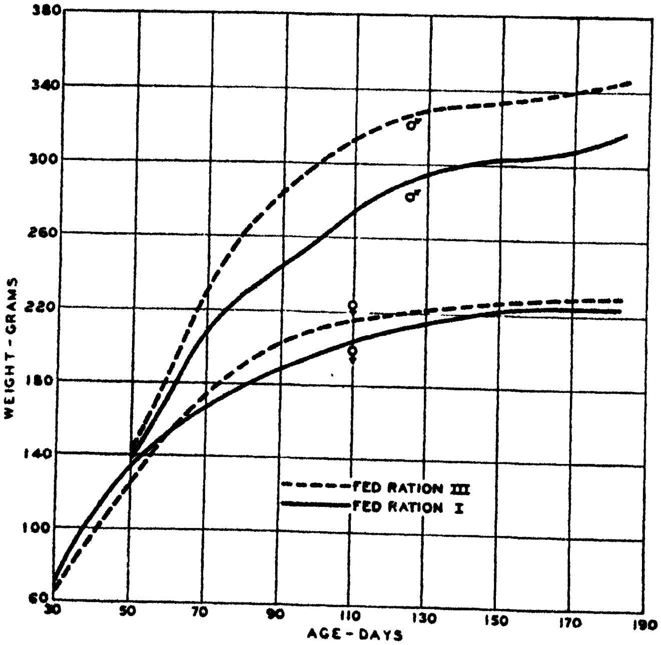

Fig. 2 Growth of rats fed ad libitum on rations I (lactose) and III (dextrin).

TABLE 2

Analyses of four pairs of rats in group I, killed at 132 to 159 days of age

	MALES						FEMALES					
	Pair 1[1]			Pair 6[1]			Pair 7[1]			Pair 11[1]		
	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Live weight	326.0	388.0		380.0	417.0		249.0	250.0		223.0	199.0	
Intestinal fill	19.0	12.6		13.4	11.8		17.3	12.9		12.4	10.8	
Net body weight	307.0	375.4	68.4	366.6	405.2	38.6	231.7	237.1	5.4	210.6	188.2	-22.4
Moisture in net body weight	171.7	182.7		214.5	218.2		127.4	122.9		131.7	102.6	
Net dry body weight	135.3	192.7	57.4	152.1	187.0	34.9	104.3	114.2	9.9	78.9	85.6	6.7
Fat in net body weight	62.2	115.0	52.8	65.7	103.6	37.9	49.1	64.9	15.8	25.3	45.3	20.0
Protein in net body weight	60.2	64.7		74.7	73.7		42.3	40.9		44.7	33.5	
Ash in net body weight	10.5	11.2		9.5	8.4		8.6	7.7		7.7	5.5	

[1] Age when killed: pair 1, 132 days; pair 6, 150 days; pair 7, 159 days; and pair 11, 146 days.

TABLE 3

Span of life of rats on lactose and sucrose rations

GROUP	PAIR	AGE OF LACTOSE-FED RAT AT DEATH	AGE OF SUCROSE-FED RAT AT DEATH	DIFFERENCE
		days	*days*	*days*
I	2	897	336	561
I	3	951	386	565
I	4	867	573	294
I	5	879	1095	-216
I	9	877	499	378
I	10	720	627	93
I	15	1040	814	226
I	16	986	1070	-84
I	17	908	687	221
II	1	785	790	-5
II	2	1000	780	220
II	3	899	676	223
II	4	992[1]	779	213
II	6	340	340[2]	
II	7	555[2]	555	
II	8	566	977[1]	-411
	Average group I	903	676	227
	Average group II	848	800	48
	Average of all	883	720	163

[1] Still alive when this table was made. They are included in averages.

[2] Died before its mate. Mate was then discarded. Neither is included in averages.

a year later than group I. Sucrose and lactose rations of the same composition as before were used, but each rat was kept in a separate cage and members of each pair, being allowed as much food as the less avid member would eat, consumed practically the same quantity. The rats of this group were started on the experiment at weaning. Weight changes are shown in figure 3, and data on life span for this group are in table 3. Post-mortem examinations revealed nothing significant. Two pairs of rats in group II were killed and analyzed, the results being given in table 4.

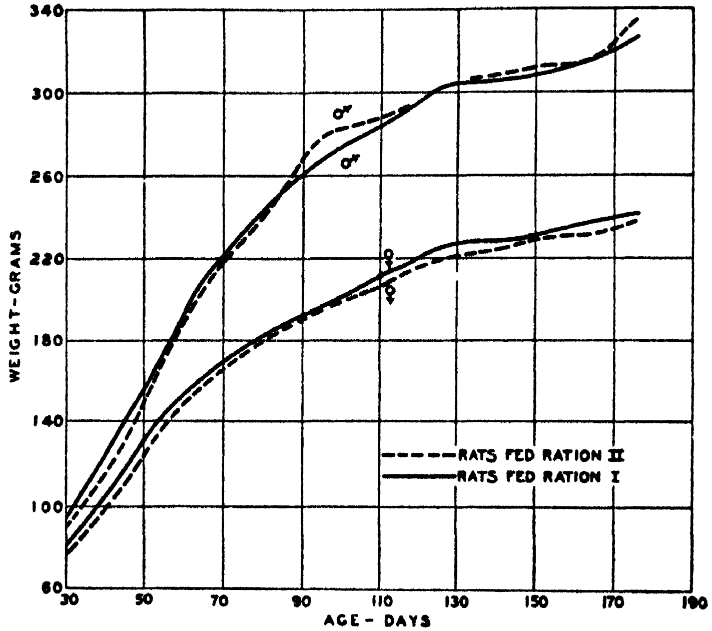

Fig. 3 Growth of rats on rations I (lactose) and II (sucrose), members of each pair were fed the same quantity.

Group III, consisting of twelve rats, was used to determine the effects of larger proportions of sucrose and of lactose in the rations—45 and 63.5 per cent. The results are shown in figures 4 and 5, the rations used being listed in table 1. The rats tolerated the large amounts of sucrose very well, but all of the rats on the high lactose rations developed diarrhea and did not gain weight satisfactorily for about 3 weeks. At the end of that time, however, they recovered from the diarrhea and began good gains, in spite of the fact that they were consuming the large amounts of lactose. In a short time, the

TABLE 4

Analyses of two pairs of rats in group II, killed at 774 days of age

	FEMALE PAIR			MALE PAIR		
	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Live weight	280.7	269.0	....	345.3	360.9	....
Intestinal fill	7.4	9.0	....	19.8	11.5	....
Net body weight	273.3	260.0	-13.3	325.5	349.4	23.9
Moisture in net body weight	153.2	141.0	....	180.6	193.7	....
Net dry body weight	120.1	119.0	-1.1	144.9	155.7	10.8
Fat in net body weight	59.7	62.5	....	71.1	80.5	....
Protein in net body weight	48.0	45.6	-2.4	58.5	62.0	3.5
Ash in net body weight	10.5	9.3	....	12.1	12.0	....

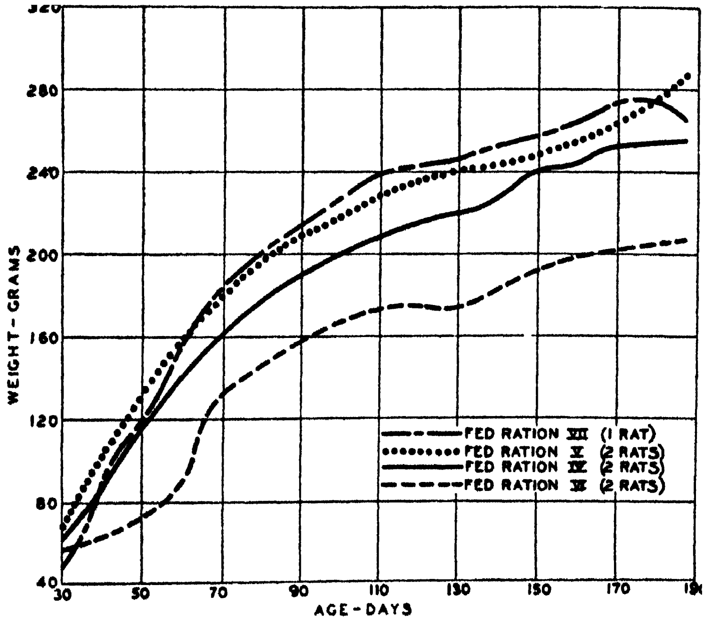

Fig. 4 Growth of female rats on rations IV (45 per cent lactose), V (45 per cent sucrose), VI (63.5 per cent lactose), and VII (63.5 per cent sucrose).

rats receiving the 45 per cent lactose ration attained practically the same weights as their partners on the same percentage of sucrose. The rats receiving the 63.5 per cent lactose ration were slower in overtaking their mates.

In the experiments with pigs, the rations listed in table 5 were used. The salt mixture contained 50.00 per cent lime-

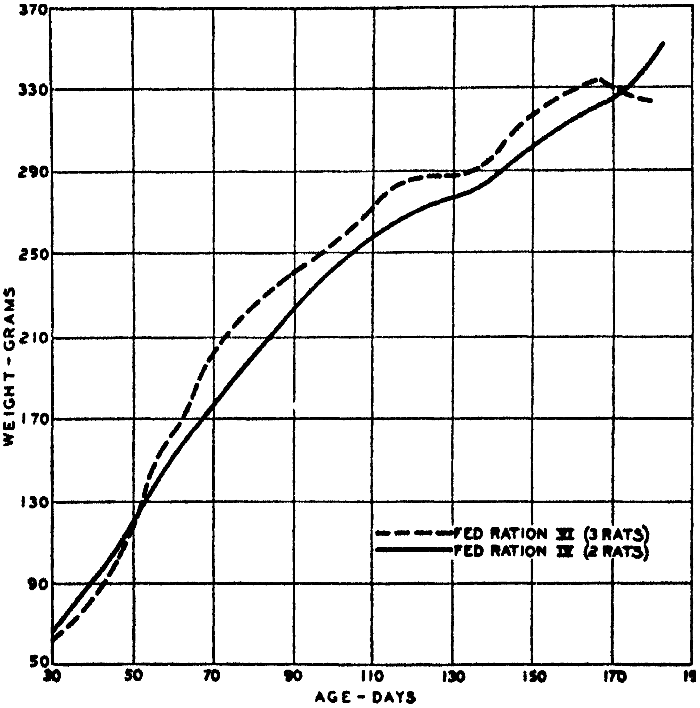

Fig. 5 Growth of male rats on rations IV (45 per cent lactose) and VI (63.5 per cent lactose).

TABLE 5

Composition of rations fed to pigs

RATION	COMPOSITION					
	Tankage	Alfalfa leaf meal	Salt mixture	Brewers' rice	Sucrose	Lactose
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Check	9	5	1	85	0	0
Sucrose	14	5	1	40	40	0
Lactose	14	5	1	40	0	40

stone, 27.97 per cent steamed bone meal, 20.00 per cent NaCl, 2.00 per cent Fe_2O_3, 0.01 per cent $CuSO_4$, and 0.02 per cent KI.

Twelve pigs were fed in four groups of three each, each pig of a group having a different ration and being fed separately. The pigs were allowed as much of the ration as the least avid member of each triplet would consume. The first week, the sugar rations contained 10 per cent sugar and 70 per cent brewers' rice; the second and third weeks, 20 per cent sugar and 60 per cent brewers' rice was fed; thereafter, the rations were fed as listed in the table. The total feeding period was 25 weeks, from February 9, 1932, to August 8, 1932. The pigs were weighed at the beginning of the experimental feeding; at the end they were weighed, slaughtered, and the edible portions of the carcasses analyzed. The results are tabulated in table 6.

DISCUSSION

During the period when the rats of groups I and II were very young, those that were fed lactose increased in weight much faster than their mates on the sucrose ration. After attaining what may be called adult age, the sucrose-fed rats of group I became heavier than their lactose-fed partners and remained considerably heavier on the average for the remainder of their lives. In group II there was a slight tendency for the adult sucrose-fed rats to be heavier than the lactose-fed rats, but the differences on the controlled feeding basis were less than those on the ad libitum feeding of group I. This is readily explainable, since the sucrose-fed rats of group I evidently consumed much more feed than their lactose-fed mates, probably because of difference in palatability, lactose being only about one-sixth as sweet as sucrose (Biester et al., '25).

Dextrin parallels sucrose rather than lactose in its effects, when fed in moderate amounts, thus indicating that supplying carbohydrate to the lower intestine for stimulation of acidophilic organisms is probably not the factor causing the differentiation in the effects of lactose and sucrose.

TABLE 6

Growth and composition of pigs fed sucrose and lactose rations

RATION FED	GROUP	LITTER AND INDIVIDUAL NUMBER[1]	BREED[2]	SEX	WEIGHTS		GAIN		FEED CONSUMED PER POUND GAIN	COLD DRESSED CARCASS	TOTAL EDIBLE MEAT	CHEMICAL COMPARISON OF TOTAL EDIBLE				FIRMNESS	REF. INDEX 40°
					Initial	Final	Total	A.D.G.				Water	Ash	Fat	Protein		
					lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*		
Control	A	304.2	DJ × Tam.	M	67	179	112	0.63	4.28	131.5	96.02	42.91	0.62	44.32	11.92	H	1.4589
	B	6.4	C.W.	F	71	189	118	0.73	5.18	146.5	108.46	40.11	0.52	48.53	11.10	MH	1.4591
	C	213.1	P.C.	F	88	186	98	0.55	5.41	147.5	105.34	48.78	0.66	37.55	13.29	MS	1.4594
	D	8.3	C.W.	F	82	212	130	0.81	4.74	168.5	122.86	46.69	0.54	39.08	13.51	H	1.4587
Average					77	191.5	114.5	0.68	4.90	148.5	108.42	44.62	0.58	42.37	12.46		1.4590
Sucrose	A	8.5	C.W.	F	62	127	65	0.37	7.15	97.5	69.22	41.32	0.58	46.95	10.28	S	1.4597
	B	6.3	C.W.	M	80	165	85	0.63	6.01	Died near the end of the experiment							
	C	213.5	P.C.	F	85	190	105	0.59	5.05	149.0	108.62	41.36	0.53	47.78	10.64	MS	1.4595
	D	304.5	DJ × Tam.	M	89	181	92	0.57	6.72	135.5	94.76	38.73	0.40	50.68	10.38	MS	1.4595
Average for 3					78.6	166	87.3	0.54	6.23	127.3	90.87	40.47	0.50	48.58	10.43		1.4596
Lactose	A	8.7	C.W.	F	59	139	80	0.45	5.79	110.0	76.12	48.95	0.63	38.41	11.90	MH	1.4589
	B	6.2	C.W.	M	88	189	101	0.63	6.03	144.0	101.32	47.49	0.57	39.03	13.54	MH	1.4594
	C	213.6	P.C.	F	85	148	63	0.36	8.57	113.0	80.08	47.93	0.67	39.66	12.34	MS	1.4594
	D	103.9	D.J.	M	84	185	101	0.63	6.13	137.0	94.62	43.56	0.68	43.94	12.05	MH	1.4589
Average					79	165.2	86.2	0.52	6.63	126.0	88.04	46.98	0.64	40.26	12.46		1.4592

[1] That is, 304.2 signifies pig no. 2 of litter no. 304.

[2] DJ × Tam. = Duroc-Jersey Tamworth cross; C.W. = Chester White; P.C. = Poland China.

The data of table 2 show clearly that the differences in weight of adult rats fed ad libitum on the lactose and the sucrose rations are accounted for practically entirely by differences in the amounts of fat present in their bodies. Definitely more fat is present in the bodies of sucrose-fed females in this group even when their body weights are no greater than those of their lactose-fed partners. These results have been confirmed by the previously mentioned work with pigs. Of interest in this connection is an observation of Kotchneff ('24) that lactose is almost entirely retained by the liver as glycogen.

As shown by table 3, the sucrose-fed rats of group I died sooner than their mates in seven comparisons out of nine. The same tendency was manifest in group II. We do not suggest any explanation of these observations on longevity.

The results of feeding the 45 and the 63.5 per cent sugar rations point to the reason for Mitchell's findings ('27) that growth on a lactose ration is comparatively poor. After the period of adaptation, during which the diarrheal condition and slow rate of growth were the most evident features of the lactose feeding, our rats not only grew satisfactorily but practically overtook the sucrose-fed rats, which had not been retarded by intestinal disturbances.

Results of the experiments with pigs show that neither the sucrose nor the lactose ration was as suitable for pigs as was the control. The fat percentages of the carcasses of the hogs fed the sucrose ration were considerably greater than those of the other two lots, this fact substantiating the results on rats in this particular. The firmness grades and the values of refractive index of the fat show that the flesh of the sucrose-fed hogs was softer than that of the hogs on either of the other rations.

SUMMARY

Lactose caused more rapid growth of young rats than did sucrose.

Adult rats became heavier on a sucrose ration than on a lactose ration when fed ad libitum, a difference attributed to greater accumulation of fat in the bodies of the sucrose-fed rats.

Pigs fed sucrose accumulated a greater proportion of fat than did pigs fed lactose.

The characteristic results obtained on rate of growth on lactose rations are evidently not to be explained on the basis of stimulation of acidophilic organisms in the lower intestine, since dextrin has not the same influence on growth as has lactose.

Lactose-fed rats, in general, lived longer than sucrose-fed rats.

Feeding of excessive quantities of lactose to rats caused diarrheal conditions and retardation of growth for a short time, after which the disadvantage was practically overcome.

It is suggested that these results may be directly applicable to human nutrition.

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BLINDNESS IN CATTLE ASSOCIATED WITH A CONSTRICTION OF THE OPTIC NERVE AND PROBABLY OF NUTRITIONAL ORIGIN[1]

L. A. MOORE, C. F. HUFFMAN AND C. W. DUNCAN
Section of Dairy Husbandry and Section of Chemistry, Michigan Agricultural Experiment Station, East Lansing

SIX FIGURES

(Received for publication November 12, 1934)

During the past 12 years the Dairy Section of the Michigan Agricultural Experiment Station has observed twenty-four cases of blindness in dairy animals used in experiments to study the dietary factors carried by good quality hay. This particular type of blindness is present in calves—apparently developing during the intra-uterine period—whose dams received rations with a poor quality roughage. The blindness may also develop in young growing dairy animals if they receive a ration containing roughage of poor quality.

This particular type of blindness is characterized by a dilated pupil even on exposure to bright sunlight and by the absence of inflammation of the external structure of the eye. In cases following birth, this type of blindness is frequently associated with partial paralysis, especially of the front quarters, weakness, a peculiar position of the head and sometimes spasms, as shown in figure 1. In young growing animals it is associated with stiffness, poor condition, and occasional spasms. There is no known therapy which will restore the eye sight in this type of blindness either in calves blind at birth or in young growing animals. This condition is closely associated with poor reproduction as first reported by Hart and co-workers ('11, '17, '20 and '24) when the dam's ration consisted of the oat plant, the wheat plant or a mixture of oat, wheat and corn plants.

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[1] Journal article no. 196 (n.s.), Michigan Agricultural Experiment Station.

In 1926, the blindness in cattle at this station was found to be due to atrophy of the optic nerve at a point where it passed through the optic foramen from the brain. Sectioning the nerve and its bony canal in 1933 showed that the constriction could be due to narrowing of the bony canal.

Cases of blindness in calves and young growing dairy animals have been reported by several investigators. Reed, Huffman and Addington ('28) reported blindness in calves and showed photomicrographs illustrating a degeneration of the optic nerve. Huffman ('28) also reported blindness in calves when the dams received wheat straw as roughage. The animals considered in the two above mentioned reports are also included in this discussion. Other reports of blindness which may be related to the blindness described in this paper are recorded by Halverson and Sherwood ('30), Converse and Meigs ('32), du Toit, Malan and Groenewald ('34), Hart and co-workers ('24), Datta ('33) and Crocker ('19). Hart and Guilbert ('33) apparently described what is undoubtedly the true vitamin A type of blindness and which is different from the type of blindness reported in this paper. Likewise, Jones, Eckles and Palmer ('26) and Mead and Regan ('31) apparently described the true vitamin A type of blindness. De

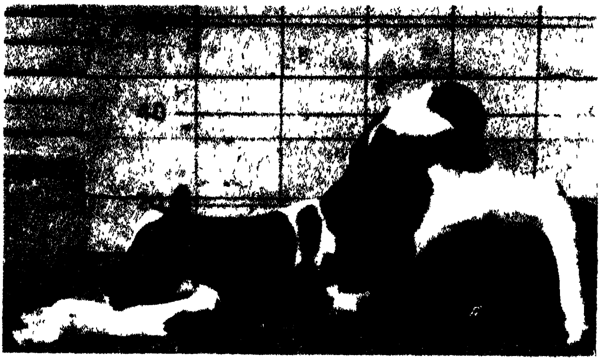

Fig. 1 Blind calf of C 200, showing paralysis of front quarters.

Schweinitz ('31) and De Schweinitz and De Long ('34) described a type of blindness due to papilledema (choked disc) which they believe may be an inherited characteristic. It is sometimes difficult to tell from the descriptions in the literature whether the type of blindness is primarily the vitamin A type, characterized by an inflammation of the external eye structures, or is the type of blindness reported in this paper in which there is no evidence of inflammation, but which may or may not be due to vitamin A deficiency.

The purpose of this paper is to report data and describe a type of blindness somewhat different from the true vitamin A ophthalmia.

EXPERIMENTAL

In 1922, work was begun at this station to study the dietary factors carried by good quality hay. This was done by feeding various types of concentrate rations with poor quality roughages and using various supplements. In this report, whenever wheat straw, hay or silage was not fed, the roughage consisted of wood shavings.

All animals were placed on their respective rations at from 3 to 6 months of age. The animals were turned outside during the day to exercise, except during inclement weather, unless otherwise noted. When the animals were kept away from sunlight they were usually turned out at night for exercise. No. 2 yellow corn was used in all rations containing corn. Iodized salt was fed. The cod liver oil used was a good grade of medicinal oil. The viosterol used was 250D. All animals received water, except when considerable milk was fed.

Accurate daily records were kept of food consumption and of any abnormalities which occurred. Blindness in calves was determined following birth by noting the reaction of the pupil of the eyes to bright light from the sun or a flash light. Blindness would first be noticed in young growing dairy animals when they started bumping into objects when they were removed from the stalls to the exercise lot.

Data pertaining to the animals are shown in tables 1 to 5, inclusive.

RESULTS

When the dams of the calves received a ration consisting principally of a grain mixture containing large amounts of corn, corn silage and wheat straw as roughage (table 1), considerable blindness and poor reproduction resulted. This ration should contain fair amounts of vitamin D, but it is low in calcium.

Six definitely blind calves were born from dams on the above ration. Four other calves were dead at delivery and one other calf died before it could be determined whether it was blind. Of the blind calves, the optic nerves of C 35's second calf and C 46's first calf were examined and showed changes. The optic nerves of C 50's first calf were also examined, but they were apparently normal.

Seven strong calves were born. In five of the seven cases of strong calves, their dams, C 46 and C 50, consumed much more silage during the succeeding gestation periods than during the first. In the other two cases the dams' rations were changed from wheat straw to timothy hay and they also consumed much more silage than during the first two gestation periods in which blind calves were produced.

The addition of 100 cc. of cod liver oil per week to the ration of animal 200 during the last 3½ months of gestation did not prevent the development of blindness in the calf. It is possible that the effects upon the fetus had developed before the cod liver oil was added to the ration or that the cod liver oil purchased in 1924 may not have been as rich in the vitamins, as that obtainable today.

When the ration of the dams consisted principally of rolled oats and oat hay, three blind calves were produced, as shown in table 2. This ration was low in calcium and in vitamin D, because the oat hay was cured in the dark, but it probably contained some vitamin A as the hay was green and leafy.

Although O 1's calf was blind, it was able to stand up and was apparently in healthy condition when sold for veal at 39 days of age. O 6's first calf was blind and paralyzed. At autopsy the optic nerves showed atrophy, as shown in

TABLE 1

Data pertaining to calves whose dams received a ration consisting principally of grain, corn silage and wheat straw

DAM NO.	CALF NO	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Grain mix	Corn silage	Wheat straw	Timothy						
		Pounds	*Pounds*	*Pounds*	*Pounds*				*Pounds*		
201	1st	10–12[1]		ad lib.		5–11–24	246	Male	73	Dead at delivery	
205	1st	14[2]				8–25–24	234	Female	38	Blind, paralyzed	Lived 1 day
200[6]	1st	12[1]		ad lib.		9–15–24	244	Female	55	Blind, finally got up	Lived 18 days
C 35	1st	7[3]	7	6–7		8– 8–26	258	Male	68	Blind, strong, stiff neck	Killed 106 days of age
C 35	2nd	12–14[4]	7–12	6–7 + ad lib.		7–14–27	260	Female	73	Blind, weak	Killed, 1 day old
C 35	3rd	8–14[4]	20–35		8–10	7–21–28	280	Male	88	Strong	
C 35	4th	8–14[4]	20–30		8–10	8–21–29	266	Female	79	Strong	Died with navel rupture next day
C 38	1st	7[4]	7	3–6		3–12–26	. . .	Female	78	Blind, paralyzed	Killed, 11 days of age
C 39	1st	7[5]	7	7		7– 6–26	. . .	Male	55	Very weak, paralyzed	Died in 19 hours B. coli infection
C 37	1st	7[5]	6– 7	7–8		8–15–26	218	. . . .	33	Dead at delivery	
C 37	2nd	12[5]	7–11	7 + ad lib.		7–13–27	246	Male	. .	Dead at delivery	
C 46	1st	7[3]	7	7		9–26–27	270	Female	70	Blind, weak	Killed 2 days of age
C 46	2nd	12–16[4]	15–25	5–7		10–15–28	276	Female	74	Strong	
C 46	3rd	8–20[4]	30	3–9		10–24–29	282	Male	100	Strong	
C 50	1st	6– 7[3]	4– 7	5–7		2–12–27	263	Male	81	Dead	
C 50	2nd	8–16[4]	12–20	5–7 + ad lib.		4–21–28	282	Male	94	Strong	
C 50	3rd	8–18[4]	20–25	5–9		4–23–29	277	Male	106	Strong	
C 50	4th	8–15[4]	20–30	5–6		8–15–30	292	Male	98	Strong	

[1] 400 pounds yellow corn
50 pounds corn gluten meal
50 pounds cottonseed meal
5 pounds sodium chloride

[2] 100 pounds of [1] plus
5 pounds wheat straw

[3] 300 pounds yellow corn
100 pounds oats
100 pounds cottonseed meal
5 pounds sodium chloride

[4] 300 pounds yellow corn
100 pounds oats
200 pounds linseed oil meal
5 pounds sodium chloride

[5] 100 pounds of [3] plus 3 per cent magnesium phosphate.

[6] Received 100 cc. of cod liver oil one day each week for last 3½ months of gestation period.

TABLE 2

Data pertaining to calves whose dams received a ration consisting principally of rolled oats and oat hay

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Rolled oats	Oat[1] hay	Cod liver oil	Grain mix						
		Pounds	*Pounds*	*cc.*	*Pounds*				*Pounds*		
O 1	1st	6	6			10–24–27	270	Female	63	Blind, able to stand up	Sold, posted 12–12–27
O 6	1st	4– 5	8			5–22–28	252	Female	47	Blind, paralyzed	
O 6	2nd	8–10	8–10	20		4–17–29	278	Male	96	Strong	
O 6	3rd			20	10–12[2]	7–14–30	270	Female	68	Strong	
O 8	1st	5– 7	5– 7			6– 3–29	272	Female	71	Blind, weak	Died 6–7–29
O 8	2nd			20–50	8–15[2]	2–23–31	268	Male	79	Active, normal	

[1] Cured in dark.

[2] 40 pounds linseed oil meal
20 pounds wheat bran
60 pounds yellow corn
60 pounds oats
4 pounds bone meal
4 pounds calcium carbonate
2 pounds sodium chloride

figure 2. O 6 was able to produce a normal calf, however, when the above ration was supplemented with 20 cc. of cod liver oil per day. This animal also produced a normal calf when she received a grain ration supplemented with 20 cc. of cod liver oil per day. O 8 received a ration of rolled oats and oat hay during her first gestation period and gave birth to a blind and weak calf. During the second gestation period O 8 received a grain mixture supplemented with 20 to 50 cc. of cod liver oil per day and gave birth to a normal healthy calf. These two cases furnish some evidence that cod liver oil carries the factor or factors necessary to prevent this type of blindness.

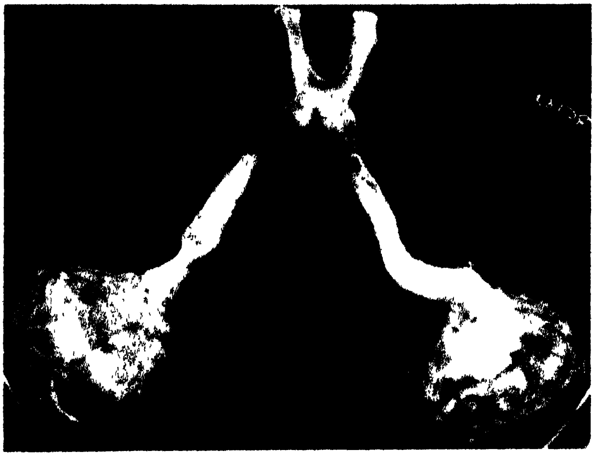

Fig. 2 Constriction of the optic nerve of O 6's first calf.

When the dam's ration consisted principally of skim milk, corn and oats, as shown in table 3, one blind and one paralyzed calf were produced. C 75 produced a normal calf when she received the above ration supplemented with a 20 to 50 cc. of cod liver oil per day. Her second calf, however, was blind and paralyzed, although she received 50 cc. of cod liver oil per day during the first month of gestation. After the first month of gestation C 75 was kept away from sunlight. At autopsy the optic nerves of C 75's second calf were found to be severely atrophied. C 83 received a ration consisting

TABLE 3

Data pertaining to calves whose dams received a ration consisting principally of skim milk, corn and oats

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION					DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Skim milk	Corn and oats	Cod liver oil	Cotton-seed meal	Wheat straw						
		Pounds	*Pounds*	*cc.*	*Pounds*	*Pounds*				*Pounds*		
C 75	1st	16	7–10	20–50			2–28–31	273	Male	82	Strong	
C 75[1]	2nd	30	16–18	50[2]			2– 6–32	243	Female	47	Blind, paralyzed	Killed 2 days of age
C 83	1st		1.5	30	8	2	10–20–31	261	Male	68	Normal	
				Ultraviolet								
C 83[3]	2nd	30	5– 8	15 min.			5– 1–33		Male	63	Normal	
C 83[4]	3rd	30–35	8–13	15 min.			3–15–34	237	Male	35	Paralyzed	Died in a few hours

[1] Kept from sunlight after first month of gestation.

[2] First month of gestation.

[3] Pasture first 6 weeks of gestation after which did not receive sunlight.

[4] No sunlight.

of cottonseed meal, wheat straw and 30 cc. of cod liver oil per day during her first gestation period. Her first calf was normal. During the first 6 weeks of C 83's second gestation she was on pasture, after which time she was kept away from sunlight, but was irradiated with a quartz mercury lamp for 15 minutes per day and received a ration consisting of skim milk, corn and oats during the rest of the period. Her second calf was also normal. During C 83's third gestation, she received the same ration and treatment as that received after the first 6 weeks of the second gestation period. The third calf, however, was paralyzed at birth and died before it could be determined whether or not it was blind. The optic nerves showed marked atrophy upon sectioning. The blood calcium and inorganic phosphorus values were markedly reduced during the last 6 weeks of gestation.

The ration of the dams shown in table 4 consisted principally of a grain mixture with various supplements. This ration contained considerable vitamin A in the form of yellow corn and corn gluten meal. It also had an optimum calcium to phosphorus ratio, but it was low in vitamin D, except when this was supplied in various forms and amounts, as shown in the table. All of the calves produced were either blind or dead at delivery, with the exception of C 172's calf which was probably killed during delivery. The optic nerves of this calf, however, showed the characteristic degeneration. All of the blind calves in this series showed the characteristic atrophy of the optic nerves. From sections of the nerves of the calves of C 108 and C 118 it was evident that the atrophy could be due to bone pressure. A cross section of the nerve within the bony canal of C 118's calf compared with a similar cross section from a normal calf at birth is shown in figures 3 and 4. Figure 5 shows a longitudinal section of the nerve of C 108's calf.

The sections were made by decalcifying the bone tissue and removing representative sections every 500 μ along the nerve where it passed through the bony canal. It is of interest to observe that the diameter of that portion of the bony canal

TABLE 4

Data pertaining to calves whose dams received a ration consisting principally of a grain mixture

DAM NO.	CALF NO	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Grain mix	Cod liver oil	Sunshine and ultra-violet	Magnesium oxide						
		Pounds	*cc.*		*gm.*				*Pounds*		
C 103	1st	10	20	None		12–12–32	247	Male	79	Blind, paralyzed	Lived 3 days
C 106	1st	11		+		5–13–33	279	Female	77	Blind, paralyzed	Killed 1 day of age
C 108	1st	13	20	None		8– 4–33	267	Male	83	Blind, strong	Killed 50 days of age
C 118	1st	12		+		11–19–33	260	Male	69	Blind, paralyzed	Lived 3 days
C 97	1st	11–13		+		10–10–32	254	Male	52	Dead	
(twins)								Male	52	Dead	
C 172	1st	11	5	None	24	4–16–34	263	Female	74	Killed in pulling	
			Viosterol 20 drops								
C 112	1st	11		None		1–26–33	157	. . . . .	. .	Dead	

[1] 50 pounds yellow corn
20 pounds oats
20 pounds yellow corn gluten meal
8.5 pounds linseed oil meal
1 pound calcium carbonate
0.5 pound sodium chloride

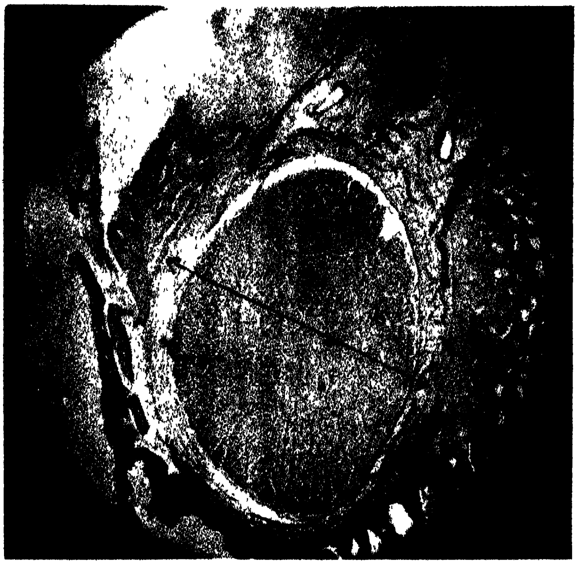

Fig. 3 Cross section of the optic nerve and bony canal of a normal calf at birth. × 16.

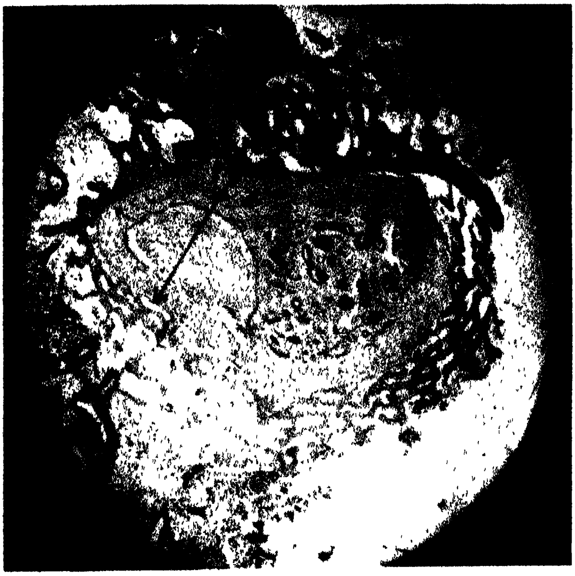

Fig. 4 Cross section of the optic nerve and bony canal of C 118's calf. Note difference in diameter of bony canal compared with the normal. × 16.

through which the nerve passed was only about one-half the normal diameter, as shown in figures 3 and 4. It can also be seen that the nerve is almost completely degenerated, and that the connective tissue surrounding the nerve and its accompanying nerves and blood vessels is considerably more dense than the normal.

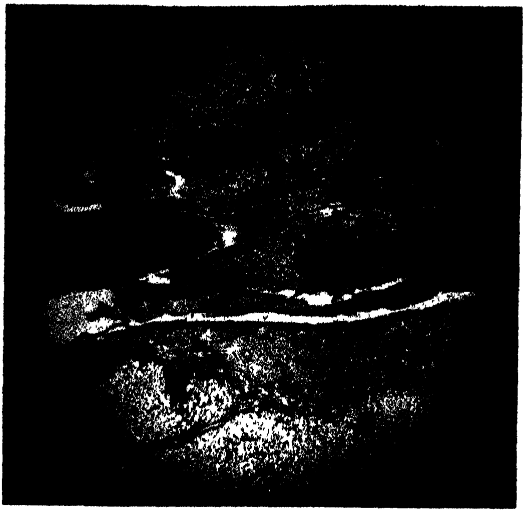

Fig. 5 Longitudinal section of the optic nerve and bony canal of C 108's calf. × 16.

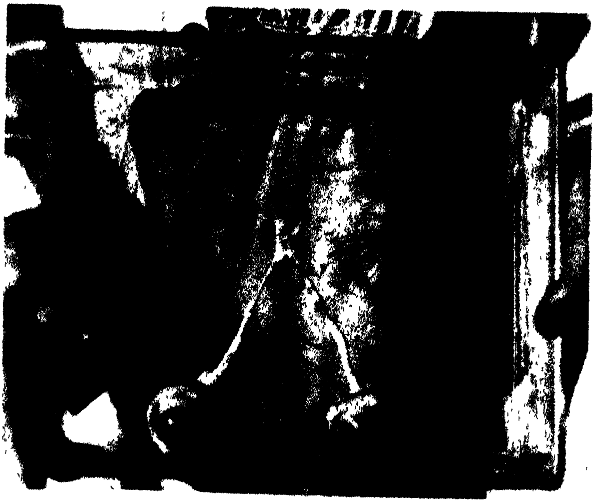

Fig. 6 Constriction of the optic nerve of C 91.

TABLE 5

Data relative to blind growing dairy animals

ANIMAL NO.	DAILY FOOD CONSUMPTION DURING PERIOD OF DEVELOPMENT OF BLINDNESS					AGE WHEN PLACED ON RATION	AGE WHEN BLINDNESS WAS FIRST NOTICED	DATE WHEN BLINDNESS WAS FIRST NOTICED	DEGREE OF BLINDNESS WHEN FIRST NOTICED
	Grain mix	Potatoes	Timothy hay	Skim milk	Corn and oats				
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Days*	*Days*		
C 20	6–8[1]	2–5	1			115	404	1– 5–25	Totally
C 21	6–8[2]	5	1			127	351	11–30–24	Partially
			Viosterol						
C 112[9]	8–10[3]		20[4] drops			94	532	5– 1–32	Partially
C 32				12–16	3–5	109	413	9– 8–25	Partially
	Corn dis. grain	Corn gluten	Wheat straw						
C 44	0.5	2–3	ad lib.	12[5]	2–2.5	89	347	3– 9–26	Totally
C 45	0.5	2–3	ad lib.	12[6]	2–2.5	120	293	1–16–26	Totally
	Cottonseed meal								
C 43	2–6		ad lib.		2–4	94	292	10–31–25	Totally
C 49	3			6[7]	2–3	91	189	11–16–25	Partially
C 80	2–5		1–3	12[8]	1–2	94	311	7– 1–29	Partially
C 91				12	4–5	98	354	1– 1–31	Totally

[1] 150 pounds hominy
100 pounds corn distillers grains
50 pounds wheat straw
25 pounds cottonseed meal
17 pounds yeast
2 pounds sodium chloride
1 pound sodium phosphate
1 pound di sodium phosphate
0.5 pound magnesium phosphate
0.5 pound magnesium carbonate
0.25 pound potassium chloride
0.25 pound potassium carbonate
20 grams iron lactate

[2] 100 pounds grain mixture [1] plus
3 pounds calcium carbonate

[3] 50 pounds yellow corn
20 pounds oats
20 pounds yellow corn gluten meal
8.5 pounds linseed oil meal
1 pound calcium carbonate
0.5 pound sodium chloride

[4] 250 D.

[5] To 189 days of age.

[6] To 187 days of age.

[7] To 133 days of age.

[8] To 206 days of age.

[9] No sunlight.

Various types of rations were used which produced blindness in young growing dairy animals, as shown in table 5. The optic nerves of C 43, C 44, C 80, C 91 and C 112 were dissected out and showed the characteristic atrophy where they passed through the bony canal. Figure 6 shows the typical condition of the nerves of C 91.

Weekly or bimonthly blood calcium and inorganic phosphorus values were obtained on the dams of all the blind calves, except animals 200, 201 and 205. Some blood determinations were also made on a few of the blind calves at the time of birth. In many cases plasma chloride, carbon dioxide and hemoglobin determinations were made. These data, however, do not reveal any outstanding abnormalities. Similar data were obtained on the young growing dairy animals shown in table 5, but the data did not reveal anything which could be correlated with the development of blindness in every case.

DISCUSSION

The evidence presented indicates that corn silage, timothy hay and cod liver oil contain some factor or factors which prevents blindness in calves due to constriction of the optic nerves. From the cases thus far studied, it is evident that when the dams receive a ration with a roughage of poor quality, the blindness in their calves is due to atrophy of the nerve at the place where it passes through the bony canal. It also seems plausible from the evidence obtained in sectioning the nerves in the bony canal in three cases, and observing the small opening through which the nerve must pass in other cases, that the atrophy could be due to the pressure of the bone upon the nerve (fig. 4). It might be argued that the nerve first degenerated and that the canal through the bone did not enlarge as growth proceeded. It is difficult to conceive, however, that the degeneration of the optic nerve, if it were due to causes other than pressure, would always occur at the same place.

Examination of the bones from several cases did not reveal any exostosis. The bony canal gave more the appearance of having had pressure applied from above which caused it to become smaller as growth proceeded. Due to the position of this particular structure, this would be the case if sufficient intracranial pressure were developed. The only evidence of intracranial pressure was that the animals were often subject to spasms on the slightest provocation. Furthermore, this type of blindness has never been noted to develop after the animal had reached a mature age, suggesting that sufficient calcification of the structures had probably taken place to resist any pressure.

Attention is also called to the possibility of a disturbed mineral metabolism related to vitamin D and rickets or to a low calcium ration. Examination of the blood calcium and inorganic phosphorus values did not reveal any correlation between blindness, low blood calcium, and inorganic phosphorus which would indicate that the animals were not suffering from rickets. All the animals were turned out to sunshine, except where indicated, yet blindness seemed to occur in almost any season of the year. When the animals were kept inside, they received ultraviolet light, viosterol or cod liver oil as a source of vitamin D, except in the case of C 75. X-rays of the ribs of many of the animals did not show a rachitic condition. The ash and mineral analyses of some of the bones were also made, but they did not reveal a rachitic condition. Therefore, it would seem doubtful that vitamin D was directly concerned in the cause of this type of blindness. The rations fed to the animals listed in tables 1 and 2 are low in calcium, but the rations fed to those listed in table 4 contained ample calcium.

Although no direct evidence has been produced, Hart and co-workers ('24), Halverson and Sherwood ('30) and Converse and Meigs ('32) believe that vitamin A is concerned in this problem where they report poor reproduction and blindness on rations with a poor quality of roughage. The type of blindness herein reported is not characterized by the external eye lesions associated with vitamin A deficiency, as reported by Mead and Regan ('31), Jones, Eckles and Palmer

('26) and Hart and Guilbert ('33). It seems doubtful that vitamin A could be concerned with such bone malformations as in the cases reported in this paper, but such an explanation might be plausible if the absence of vitamin A, in some indirect way, raised the intracranial pressure. Most of the rations used contained variable amounts of vitamin A from yellow corn. The rations shown in table 4 contained 50 per cent yellow corn and 20 per cent gluten meal. Corn gluten meal contains considerably more vitamin A than yellow corn. Even with the addition of 20 cc. of cod liver oil to the rations of C 103 and C 108 blind calves were produced. In every case where normal calves were produced on these rations, however, vitamin A had been added, so that it cannot be definitely ruled out. It is doubtful whether any of the rations contained as much vitamin A as is contained in 6 pounds of good quality alfalfa hay. Work is now in progress in which vitamin A in the form of carotene is being added to the ration in amounts equivalent to the vitamin A content of 10 pounds of alfalfa hay.

The question of storage of the dietary factor or factors concerned in this type of blindness must also be considered. Two mature cows from the regular dairy herd, not previously mentioned in this paper, and pregnant for 6 weeks, were placed on a ration of skim milk, corn and oats with shavings for roughage. These cows had normal calves at the end of a proper gestation period. C 83 (table 3) apparently received a sufficient amount of the factor or factors from 6 weeks on pasture at the beginning of the second gestation period to produce a normal calf.

This type of blindness is usually associated with poor reproduction, such as weak premature calves and frequently retained placentas. The calves were usually paralyzed in the front quarters, were too weak to suckle and sometimes exhibited spasms. The blindness may not always be associated with poor reproduction. However, O 1's, C 108's and C 35's first calves were fairly strong and active, although they were carried only 270, 267 and 258 days, respectively.

The blindness, whether it is present in the calf following birth or in the young growing dairy animal, appears to be due to identical factors, since in both cases there is a constriction of the optic nerve at the place where it passes through the bony canal. This type of blindness is more easily produced in calves during the intra-uterine period than in young growing dairy animals. It can be produced regularly in calves in utero provided the storage of the factor or factors is exhausted before gestation sets in, but it cannot be produced regularly in young growing dairy animals. Probably with more information concerning the factors involved, it can be produced with more regularity in young growing animals.

De Schweinitz ('31) and De Schweinitz and De Long ('34) report some excellent work on blindness in Guernsey calves apparently due to papilledema. Evidently they have not observed cases of constriction of the optic nerves as reported in this paper. They do discuss blindness, reported by others, due to atrophy of the optic nerve, which is apparently identical with the type of blindness reported in this paper, and show a photograph (fig. 16 in their last paper). The type of blindness which they have noted may be a different stage of the type herein reported. De Schweinitz indicated the possibility that the blindness observed in his cases may be inherited. In the work reported in this paper all of the cases have been produced in Holstein calves and we have never noticed blindness in the herd unless roughage was a limiting factor. Furthermore, this type of blindness is associated with paralysis and weakness at birth and poor reproduction, which De Schweinitz has apparently not noted in his cases.

Crocker ('19) described what is apparently the same type of blindness reported in this paper. He reported that the bone grew in such a manner as to stenose the canal and pressed the optic nerve within it.

The symptoms of cottonseed meal injury in cattle appear to be identical with the symptoms associated with this type of blindness. There is also the possibility that the curative factor or factors are identical. The evidence presented in this paper indicates that a deficiency of vitamin A in the ration may not be the causative factor involved in cottonseed meal injury.

The authors have not observed blindness among calves under farm conditions. There is a possibility, however, during years of hay shortage, that cattle may suffer due to a lack of the factor or factors carried by hay which are necessary for good reproduction and health.

SUMMARY AND CONCLUSIONS

1. Twenty-four cases of a nutritional type of blindness have been described which are apparently different from the true vitamin A type of blindness.
2. The blindness is observed in calves following birth and in young growing dairy animals when a ration containing poor quality roughage has been fed.
3. It is characterized by a dilated pupil and by the absence of inflammation of the external structures of the eye.
4. It is frequently associated with paralysis, weakness, spasms and poor reproduction denoted by premature births and retained placentas.
5. The blindness is due to atrophy of the optic nerve where it passes through the optic foramen, apparently because of bone pressure.
6. A deficiency of vitamin A may or may not be the cause of this type of blindness.
7. The evidence indicates that vitamin D is not the major factor in this type of blindness as the blood picture is not significantly different from normal.
8. The evidence further indicates that a deficiency of calcium is not directly concerned in this type of blindness.
9. Corn silage, timothy hay and cod liver oil contain the factor or factors necessary to prevent this type of blindness.

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INTESTINAL STASIS IN LOW MINERAL DIETS

ELIZABETH CHANT ROBERTSON AND M. ELIZABETH DOYLE
Department of Pediatrics, University of Toronto, and the Hospital for Sick Children, Toronto, under the direction of Alan Brown

FOUR FIGURES

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In the course of experiments on the resistance of rats fed a mineral poor diet to oral infection with a paratyphoid bacillus, some of the rats were given carmine in order to learn how soon the organisms were excreted. It was noticed that the rats passed carmine-colored feces for many days and it was thought advisable to investigate this intestinal stasis further.

ANIMALS AND BASAL DIETS

Litters of albino rats were divided a day or two after weaning into two or more groups. One group was then fed an adequate diet, and the other group was given the same diet minus the salt mixture and NaCl. The two diets were constituted as follows:

	Adequate diet Per cent	*Mineral deficient diet* Per cent
Cornstarch	59	64
Purified casein (treated with dilute acetic acid and also hot alcohol)	18	18
Crisco	10	10
Cod liver oil	2	2
Dried brewer's yeast	6	6
NaCl	1	..
Osborne and Mendel's salt mixture	4	..

The mineral deficient diet was not mineral free, as the casein contained some P and Ca, and the yeast contained a total ash of about 5.6 per cent. The Ca, P, Fe and Cu contents of the diets were determined and were found to be as follows:

Elements	*Mineral deficient diet*	*Adequate diet*
Ca	0.0794 gm. per cent	0.4294 gm. per cent
P	0.1176 gm. per cent	0.3276 gm. per cent
K	Not determined (call x)	x + 0.52 per cent
Fe	0.0062 gm. per cent	0.0252 gm. per cent
Cu	A trace	A trace

As is shown in figure 1, the rats fed the mineral deficient diet grew very slowly, whereas those fed the adequate diet increased rapidly in weight. The animals that had been kept on the deficient diet for 4 weeks often squeaked a great deal when touched, frequently bit anyone who handled them, which is very unusual with our normal rats, and sometimes dragged their hind legs, although they were still able to move them.

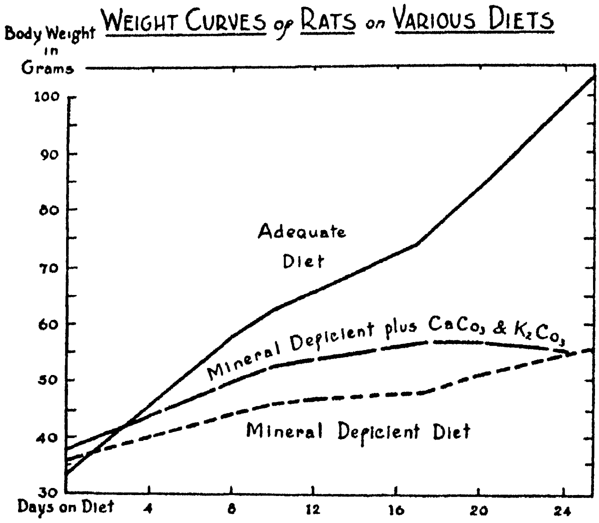

Figure 1

A few bone ash determinations and x-ray photographs were made which showed that the bones of the rats fed the mineral poor ration were osteoporotic and low in ash, whereas those of the controls were normal. The polycythemia which Swanson and Smith ('32) observed in rats fed a diet very deficient in minerals was confirmed.

Method of administering carmine. After a certain period on the diet, usually 4 weeks, the rats were starved for 16 to 17 hours and then fed by pipette about 6 mg. of carmine in 0.2 cc. of simple syrup. The same batch of carmine was used throughout. The rats were kept in individual wire mesh bottomed cages, usually for some time before feeding the carmine, and the feces were collected on paper which was placed beneath the mesh out of reach of the rats.

The first collection of feces was made 24 hours after the carmine feed and from then on daily. The pellets were examined in a little water and crushed with forceps, and if the distinctive red color was not evident they were shaken up with 3 to 4 cc. of water. This suspension sometimes appeared pink when no carmine had been seen in the individual pellets. If the carmine could not be positively detected by these means, the suspensions were examined under the microscope, using the high dry lens. Under this magnification the carmine showed up very brilliantly as cerise particles with sharp corners. When no carmine was found, at least three big drops were examined, and the carmine was not considered entirely excreted unless two such examinations at 24-hour intervals were negative. In a few animals the feces were further examined for several more days and were found to be free from carmine, thus establishing the accuracy of the end point.

We have found no evidence as yet, either by examining the mesentery under the microscope or by cutting open the kidneys, that carmine is absorbed from the gut, and the urine apparently did not contain any of it.

Experiment 1. Eight rats were placed on the mineral deficient diet and eight on the adequate diet and after 4 weeks, when the former weighed on the average 75 gm. and the latter 112 gm., the rate of excretion of the carmine was determined. The results are shown in figure 2. The carmine appeared 24 hours after it was administered, and the rats fed the adequate diet excreted carmine for from 3 to 7 days, whereas those fed the mineral deficient ration showed carmine in their feces for from 12 to 21 days. The fecal pellets of those fed the deficient diet at all times contained a lower concentration of carmine than those of the animals fed the adequate diet. The marked intestinal stasis shown by the rats fed the mineral deficient diet has been confirmed in over seventy-five other rats. A corresponding number of adequately fed controls failed to show the stasis.

Experiment 2. Three rats were kept on each of the two diets for 4 weeks and at the end of this time when those on the

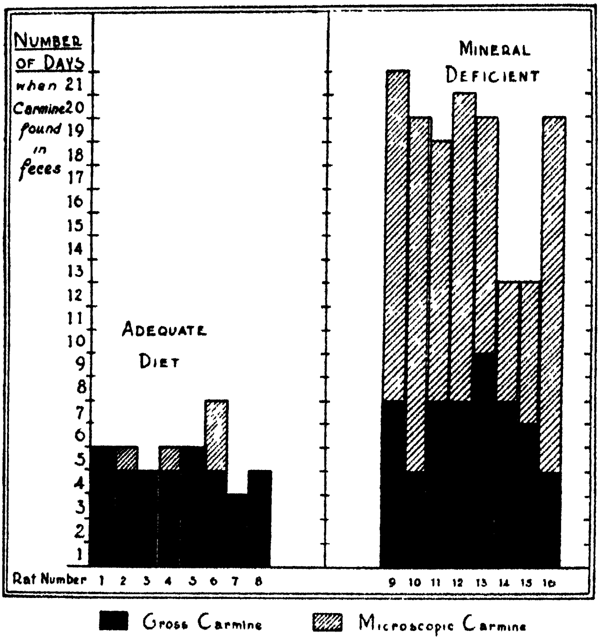

Fig. 2 The much slower excretion of carmine by rats fed the mineral deficient diet as compared with those fed the adequate diet.

adequate diet weighed 135, 117, and 112 gm., respectively, and those on the mineral deficient diet 58, 52 and 47 gm., they were fed the carmine. The rats on the adequate diet excreted carmine for 3 to 5 days, and those on the mineral deficient diet for 10 to 17 days (fig. 3, columns 1, 2, 3 and 7, 8, 9).

In this experiment the weight of food eaten and of the feces excreted daily by each rat during the period in which the carmine was being excreted was also determined. The feces were dried for 48 hours on a hot radiator before they were weighed. The results are shown in table 1.

The food intake and the weight of the feces of rat 141 were low, because its water bottle did not run properly from the third to the fifth day of the test. Despite this fact it eliminated the carmine as rapidly as rat 140 which was fed the same diet.

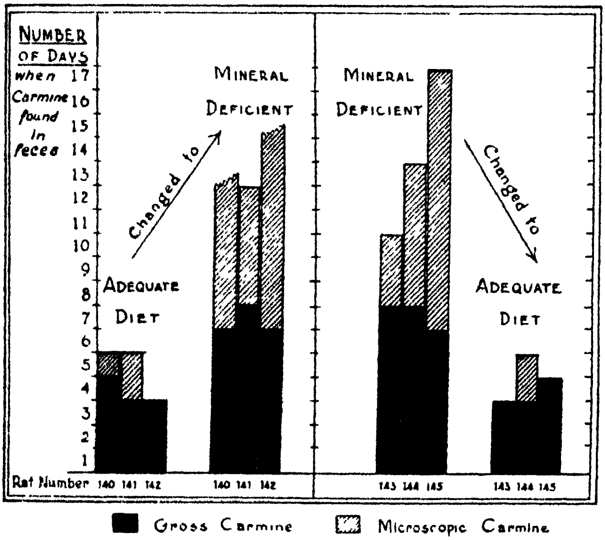

Fig. 3 The effect of alternating the deficient and the adequate diets on the rate of excretion of carmine.

TABLE 1

RAT NO.	DIET	NUMBER OF DAYS CARMINE EXCRETED	TOTAL FOOD EATEN WHILE EXCRETING CARMINE	TOTAL DRIED FECES CONTAINING CARMINE
			gm.	gm.
140	Adequate	5	31.5	2.177
141	Adequate	5	36.9	1.051
142	Adequate	3	42.1	1.205
143	Mineral deficient	10	68.6	3.446
144	Mineral deficient	13	87.8	1.905
145	Mineral deficient	17	84.7	1.807

The daily food intake of the rats fed the mineral poor ration was, as one would expect from their much smaller body weights, somewhat less than half of that eaten by the rats on the adequate diet. One might guess that it would take the same amount of food to carry away all of the carmine no matter what the diet was. Such, however, does not follow from this experiment as the rats fed the mineral deficient diet had to eat a great deal more food and excrete more feces before they got rid of all the carmine than did those fed the adequate ration.

Experiment 3. Effect of alternate deficient and adequate diets. Rats 140, 141 and 142, which had been fed the adequate diet for 43 days, were placed on the mineral deficient diet, and after 34 days on it, when they weighed, after 18 hours' starvation, 159, 136 and 137 gm., they were fed carmine again. Similarly, the rats which had been fed the mineral deficient diet for 52 days were changed to the adequate diet, and after 25 days on it, when they weighed 171, 163 and 113 gm. (starved), they were again fed carmine. All of these rats were consequently of approximately the same weight. The weight curves of these rats are shown in figure 4. Even after

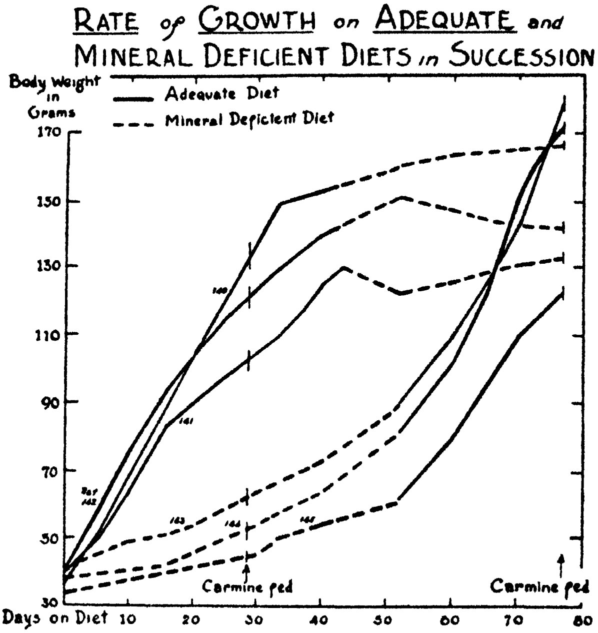

Figure 4

52 days on the deficient diet, during which time very little growth occurred, the subsequent feeding of the adequate diet led to rapid gains (rats 143, 144, 145). The rates of excretion of the carmine after the diets had been changed are shown in columns 4, 5, 6 and 10, 11 and 12 of figure 3. Marked stasis was shown by the rats, formerly on the adequate diet, which had been fed the deficient diet for 5 weeks, and no stasis in the rats which had been on the adequate diet for 4 weeks, although they had previously shown stasis on the deficient diet. Unfortunately, the exact day on which rats 140 and 142 stopped excreting carmine was not determined. It was some time after 12 and 14 days. The results of this experiment rule out the factor of body weight, as the stasis shown by rats 140, 141 and 142 (on mineral deficient diet) was as great as usual, although they weighed as much as the adequately fed animals. This experiment also shows that the deleterious effects of the mineral poor diet did not permanently injure the rats.

TABLE 2

Carmine excretion

RAT NO.	ON MINERAL DEFICIENT DIET		AFTER 7 DAYS ON ADEQUATE DIET	
	Gross	Microscopic	Gross	Microscopic
	Days	*Days*	*Days*	*Days*
17	8	10	3	7
18	7	11	3	6

Experiment 4. The above experiment was repeated in a somewhat modified form. The rate of excretion of carmine was determined in two rats which had been fed the mineral deficient diet for 5 weeks. After 3 weeks more on the deficient diets the rats were given the adequate diet for 1 week and carmine was again fed.

The results of this test in table 2 show that the stasis is much improved, although not entirely relieved by 1 week's feeding of the adequate diet.

Experiment 5. To further test out the effect of body weight on the rate of excretion of the pigment, we fed carmine to fifteen young rats which had been on the adequate diet for a week and which weighed about as much as rats that had been fed the mineral deficient diet for 4 weeks. No macroscopic carmine was seen in this lot after 3 days, and in four rats it was not evident to the naked eye after only 2 days. When the feces were examined through the microscope, carmine was usually not found for more than 4 days after the carmine feeding. Occasional rats were free after 2 or 3 days, and three showed carmine on the fifth or sixth day. In other words, small normal rats excrete the carmine as rapidly or possibly more rapidly than the large normal animals and the slower elimination in the mineral deficient rats of experiment 1 was not due to their small size. These results do not correspond with those of Gross ('24), who suggested that small rats excreted charcoal more slowly than large rats.

Experiment 6. As a lack of the vitamin B complex causes intestinal stasis, it was thought that possibly the rats fed the mineral deficient diet were not able to utilize this vitamin, which was given as 6 per cent dried yeast, as well as the adequately fed controls. Therefore, 66 mg. of a vitamin B concentrate made from yeast and wheat germ was fed to the rats in addition to the usual mineral deficient and adequate diets. This amount of concentrate should contain, according to the manufacturers,[1] twice as much vitamin B_1 and as much vitamin B_2 as the rats should require daily. After 4 weeks on the diets, when the adequate rats weighed 100, 146, 80, 110 and 119 gm., and the mineral deficient 112, 55, 75 and 85 gm., the carmine was fed.

From table 3 it is seen that the rats fed the deficient diet with the additional vitamin B concentrate excreted the carmine just as slowly as the deficient rats of the preceding experiments.

The concentrate contained no Ca, Fe or Cu, but there was about 0.4 mg. P in the amount given daily. The amount of phosphorus in the diet was, therefore, almost doubled (raised to 0.218 per cent) without improving the stasis. It is doubtful, therefore, if still further additions of phosphorus would improve the elimination.

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[1] Mead Johnson & Co.

Experiment 7. Although it was not suspected that Osborne and Mendel's salt mixture would have any laxative effect, it was thought wise to substitute another standard salt mixture. Therefore, in this experiment the rats fed the adequate diet received 4 per cent of McCollum's salt mixture no. 51, which contains the following ingredients:

TABLE 3

The effect of the addition of vitamin B concentrate. Carmine excretion

ON MINERAL DEFICIENT DIET PLUS VITAMIN B COMPLEX			ON ADEQUATE DIET PLUS VITAMIN B COMPLEX		
Rat no.	Gross carmine	Microscopic carmine	Rat no.	Gross carmine	Microscopic carmine
	Days	*Days*		*Days*	*Days*
33	7	10	28	3	5
34	11	20	29	4	4
35	8	11	30	4	6
36	11	17	31	4	6
			32	4	4

	Grams
$CaCO_3$	15
K Cl	10
NaCl	5
$NaH CO_3$	7
Mg O	2
Ferric citrate	5
$KH_2 PO_4$	17

It contains no S, I, Mn, F and Al which are all present in the Osborne and Mendel salt mixture. The mineral deficient diet remained as before. The evacuation of the carmine was just as rapid in the rats fed the adequate diet containing the no. 51 salt mixture as in the rats fed the original adequate diet. One would therefore infer that a deficiency of S, I, Mn, F or Al was not responsible for the stasis. It was also found that Osborne and Mendel's salt mixture in ʒ iii to ʒ iv doses was not laxative for an adult woman.

Experiment 8. The 1 per cent of NaCl was left out of the adequate diet in one experiment, and after 4 weeks on this diet the carmine excretion was tested as before. The rats fed this diet excreted the carmine as rapidly as the controls which were fed the usual adequate diet. Consequently it would appear that the presence of NaCl does not affect the intestinal motility.

TABLE 4

Adequate diet for 8 weeks

FED CARMINE AND KILLED AFTER	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5
	24 hours	48 hours	72 hours	96 hours	168 hours
Stomach	—	⊕	—	—	—
Duodenum	—	—	—	—	—
Jejunum	—	—	—	—	—
Ileum	—	—	—	—	—
Cecum	+++	+	—	—	—
Colon	++++	+	⊕	—	—

Mineral deficient diet for 8 weeks

FED CARMINE AND KILLED AFTER	RAT 6	RAT 7	RAT 8	RAT 9	RAT 10
	24 hours	48 hours	72 hours	96 hours	168 hours
Stomach	+++	++	+	++	—
Duodenum	—	+	—	—	—
Jejunum	++	+	+	++	—
Ileum	+++	++	+	++	—
Cecum	++++	+++	++	+++	⊕
Colon	++++	+++	++	+++	⊕

+ = Gross carmine. ⊕ = Microscopic carmine only.

Experiment 9. In this experiment rats which had been fed the regular adequate diet, and others which had been given the mineral deficient diet for 8 weeks were fed carmine and then one of each group was killed daily and the intestines were examined for carmine. The results are shown in table 4. The carmine was rapidly moved through the intestinal tract by the rats subsisting on the adequate diet. On the other hand, 4 days after the administration of the carmine the rats fed the deficient diet showed macroscopic carmine in considerable quantities all through the intestine. After 7 days there was more carmine in the rat fed the mineral poor ration than in the rat on the adequate diet that was killed 4 days previously. This experiment again shows the marked stasis present in the rats fed the mineral deficient diet. The stasis seems to involve the whole intestinal tract, although it is especially evident in the cecum and colon.

Weight of the intestines and contents. Rats which had been fed the diets for varying periods of time were killed by ether, and after a ligature of thin thread had been put around the pylorus and another around the ileocecal valve, the whole intestine with as little mesentery as possible was removed and weighed at once. The small intestine was then weighed separately.

TABLE 5

Average weights of intestines and contents

BODY WEIGHTS	ON MINERAL DEFICIENT DIET			ON ADEQUATE DIET		
	Cecum and colon	Total intestines	Number of guts weighed	Cecum and colon	Total intestines	Number of guts weighed
gm.	*gm.*	*gm.*		*gm.*	*gm.*	
51- 60	1.704	4.378	1	1.583	4.692	1
61- 70	2.544	5.672	4	1.642	5.609	7
71- 80	2.694	5.990	4	1.694	5.196	2
81- 90	2.412	6.204	4	1.953	5.979	3
91-100	2.690	6.659	3	....	....	..
101-110	2.782	6.680	1	1.939	5.216	1

When the average total intestine weights of rats of closely similar body weights (table 5) were compared, it was found that those of the rats on the mineral deficient diets were heavier than those of the animals on the adequate diets, with the exception of the two smallest rats. The cecums and the colons weighed considerably more in the rats on the deficient diet in every case. In other words, definite stasis was evident in the cecum and colon of the rats fed the mineral poor diet. The difference is probably greater than is evident from these figures, as the weight of the intestinal wall is probably about the same in both groups of rats and is responsible for a large part of the total weight. As the food normally passes very rapidly through the small intestine, one would expect a priori that the stasis would occur in the large bowel.

Gross appearance of the intestinal tract. The guts of the rats which were killed in experiment 9 were briefly examined with the naked eye for gross abnormalities. No definite dilation was seen in the upper intestines or the cecums of the rats fed the mineral deficient diet. The colons of the deficiently fed rats were, however, more dilated than those of the controls, the walls of the colons were also thinner and more friable, the haustra were more prominent especially near the cecum, and the fecal matter was more plentiful and softer. This increased size of the colons was all the more remarkable when one noted that all the rats fed the mineral poor diet, except the first one killed, weighed about half as much as the rats on the adequate diet.

Attempts to alter the rate of excretion by adding various elements

In these experiments, which are not yet completed, rats were fed the mineral deficient diet with the addition of various salts for 4 weeks and after that interval the rate of excretion of the carmine was determined. Control rats fed the mineral deficient and the adequate diet were included in each experiment.

The addition of 1.1 per cent NaCl to the mineral deficient diet, which provides about as much NaCl as is present in the adequate diet, caused very little change in the rate of excretion; in a few rats it was a little faster, in others it was somewhat slower.

When 1 per cent of $CaCO_3$ was added to the mineral deficient diet, very little if any improvement in the intestinal stasis occurred. The diet after this addition contains 0.479 per cent of calcium, which is slightly higher than that of the adequate ration.

At present the effect produced when 1 per cent K_2CO_3 is added to the mineral deficient diet is being investigated. The results to date have been contradictory. This amount of K_2CO_3 compensates for the K present in the adequate diet.

When 1 per cent $CaCO_3$ and 1 per cent K_2CO_3 were added to the deficient ration, the rats excreted the carmine as rapidly as the controls fed the adequate diet. This experiment has been repeated four times and all of the twenty-seven rats which were fed this diet showed no stasis. When 0.17 per cent $MgCO_3$ was added as well, to compensate for the Mg present in the adequate diet, the results were the same as when both $CaCO_3$ and K_2CO_3 were added, i.e., there was no stasis.

The total weights of the intestines and contents of the rats fed the mineral deficient diet $+ CaCO_3$ $+ K_2CO_3$ were decidedly less than those of rats fed the deficient diet alone, and also less than those fed the adequate ration. This adds confirmation to our previous finding that the simultaneous addition of $CaCO_3$ and K_2CO_3 to the mineral deficient diet relieves the stasis.

In order to determine whether K_2CO_3 had a laxative effect per se, 1 per cent of this salt was added to the adequate diet. This approximately doubled its K content. The rats fed this ration excreted the carmine at the same rate as rats fed the regular adequate diet. In other words, the K_2CO_3 did not act as a laxative. These findings were confirmed in a second test. When the amount of the salt mixture was raised to the high amount of 8 per cent the carmine was excreted a little more rapidly than it was by the controls fed the usual adequate diet.

In the course of these experiments there was some evidence that hot weather leads to a decrease in the speed of excretion, both in rats fed the adequate diet and in those fed the mineral poor ration. This is being checked further.

DISCUSSION

The mineral poor diet used in these experiments had in all probability a much lower mineral content than even the poorest human dietaries, but it is possible that the long-continued consumption of a diet partially deficient in these factors may also lead to intestinal stasis. Constipation is a very prevalent malady among civilized peoples, and it is also a fact that many of the highly processed food-stuffs which are much favored, such as white flour and sugar, are very low in minerals. Approximately 50 per cent of the total calories in an average diet are derived from these two sources, and in the diet of the poor the percentage is often higher. In addition, the common method of boiling vegetables in which the cooking water is discarded no doubt leads to great losses of the mineral salts, especially of those that are highly soluble, such as potassium. Keller ('34) states that from three-quarters to five-sixths of the salt content of thoroughly boiled potatoes is lost when the water in which they are cooked is thrown away. Over indulgence in table salt, which is a fairly common habit, also increases the potassium loss. The combination of all these factors may perhaps play a part in the production of constipation.

In these experiments we have not yet established conclusively that both potassium and calcium salts must be added simultaneously to the deficient diet to prevent the stasis that would otherwise occur, but this would appear to be the case. It is interesting to note that numerous investigators working on other physiological problems have emphasized the importance of the K/Ca balance in maintaining the well-being of animals. On looking through the analyses of foods, one is struck by the fact that practically all of the laxative foods fed to young children are very high in potassium. Many of them contain much roughage, but this is not always the case, as, for example, with prune juice. It is not impossible that this high potassium content is a factor in their laxative effect, especially as the calcium intake is high. Again, it is well known that human milk is more laxative than cow's milk. This may possibly be due to its higher K/Ca ratio (v. Wendt, '25). Further work is being done on these very practical problems.

SUMMARY

1. Young rats fed a diet low in minerals, but adequate otherwise, show a great delay in the excretion of 6 mg. of carmine. In other words, they have a marked intestinal stasis. Control animals fed the same diet plus the minerals do not show this stasis.

2. Additional vitamin B complex does not relieve the stasis of the mineral deficient animals.

3. The simultaneous addition of $CaCO_3$ and K_2CO_3 to the mineral poor ration seems to relieve the stasis.

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A COMPARISON OF THE ANTIRACHITIC POTENCY OF COD LIVER OIL AND IRRADIATED ERGOSTEROL ON A CURATIVE AND PREVENTIVE BASIS [1]

WALTER C. RUSSELL, M. W. TAYLOR AND D. E. WILCOX [2]
Department of Agricultural Biochemistry, New Jersey Agricultural Experiment Station and Rutgers University, New Brunswick

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It is well known that antirachitically equivalent amounts of the antirachitic factor in the form of cod liver oil and irradiated ergosterol, as measured by a curative assay with the white rat, are not equally effective when used in a preventive manner with the chicken (Massengale and Nussmeier, '30; Mussehl and Ackerson, '30; Steenbock et al., '32; Bethke et al., '33, and unpublished data from this laboratory). The purpose of this investigation was to compare the antirachitic potency of cod liver oil and of irradiated ergosterol upon a curative and preventive basis, using the white rat as the test animal. Such a comparison was of interest in connection with investigations of these two sources of the factor when the chicken was used as the experimental subject. Studies with the chicken have been made, in practically every instance, upon a preventive basis and the quantities of cod liver oil and irradiated ergosterol used have been determined by curative assays made with the white rat. Hence the question was raised as to whether a preventive assay of both sources of the factor, made with the rat, would show results in agreement with a curative assay with this species, or whether they would be in agreement with the results of preventive studies with the chicken.

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[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

[2] Acetol Products Fellow. This investigation was conducted under a fellowship grant from Acetol Products, Inc., New York.

EXPERIMENTAL

For the preventive studies young rats, 55 to 60 gm. in weight, were placed upon the experimental regime at 23 to 28 days of age. Litter representatives were assigned to each of the two groups receiving the two sources of the factor. The supplements listed in table 2 were incorporated in 50 gm. of the Steenbock 2965 ration as in a curative assay. Successive 50-gm. portions were supplied throughout the experiment, a portion being consumed in about 7 days. After 40 days of feeding, the blood was drawn from the heart, under anesthesia, for the determination of inorganic P (Briggs, '24) and the femurs removed for ash determinations. Individual determinations were made of P in whole blood and of bone ash of the right femur (Russell and Massengale, '28).

The curative assay, using the line-test method, was conducted essentially as outlined by the Wisconsin Alumni Research Foundation. Young rats of the same age and weight range as those employed for the preventive study were depleted in 23 or 24 days and then carried through the 10-day test period. The supplements were mixed with 50-gm. portions of the Steenbock 2965 ration, a portion being consumed during the first 6 or 7 days of the test period.

DISCUSSION

In table 1 the line test responses at the unit levels are shown for the two sources of the antirachitic factor, at the beginning and at the end of the preventive experiment. The unit amounts or multiples thereof were used in the preparation of the 50-gm. portions of supplemented ration in the preventive study.

A comparison of the bone ash percentages, table 2, reveals no significant difference between the responses to the two sources of the antirachitic factor. The value, 38.43 per cent, for 0.5 unit of irradiated ergosterol is lower than that for

TABLE 1

Curative assay of irradiated ergosterol and cod liver oil. Responses at unit levels

DATE OF ASSAY	IRRADIATED ERGOSTEROL		COD LIVER OIL	
	Unit level	Response	Unit level	Response
	mg.		*mg.*	
October, 1932	0.0008	+(++) +, +, +, + ± —, —	45	+(++), +(++) +, +, + ±, ±
August–September, 1933	0.0008	+++ +(++), +(++) +, +, +, + ±	45	++ +(++), +(++) +, +, + ±(+) ±

NOTE: + equals a narrow continuous line. The unit level is the smallest quantity of material which causes 60 per cent of the responses to be + grade or slightly better, including one-half of those of ±(+) grade.

TABLE 2

Comparison of irradiated ergosterol and cod liver oil on a preventive basis. Summary of food consumption, gains in weight, blood inorganic phosphorus values and bone ash percentages. Duration of test period—40 days

SUPPLEMENT IN 50 GM. OF FOOD	RAT UNITS, CURATIVE BASIS	NUMBER OF ANIMALS	AVERAGE DAILY FOOD CONSUMPTION	AVERAGE DAILY GAIN IN WEIGHT	INORGANIC P OF WHOLE BLOOD			BONE ASH OF RIGHT FEMUR		
					Per 100 cc.	Probable error	Coefficient of variation	Value	Probable error	Coefficient of variation
mg.			*gm.*	*gm.*	*mg.*		*per cent*	*per cent*		*per cent*
Irradiated ergosterol										
0.0004	0.5	10	6.5	1.2	2.2[1]	± 0.036	7.29	38.43	± 0.79	9.76
0.0008	1.0	10	7.5	1.3	2.9[1]	± 0.036	5.45	43.80	± 0.74	7.98
0.0016	2.0	10	7.9	1.4	3.0	± 0.041	6.33	46.76	± 0.39	3.86
0.0080	10.0	8	7.6	1.3	3.5	± 0.035	3.90	51.18	± 0.43	3.60
Cod liver oil										
22.5	0.5	10	7.8	1.5	2.6[1]	± 0.044	5.09	41.38	± 1.00	11.40
45.0	1.0	10	7.5	1.4	3.2	± 0.053	7.78	44.26	± 0.79	9.76
90.0	2.0	10	7.5	1.3	3.5	± 0.050	6.71	46.15	± 0.74	7.98
450.0	10.0	8	7.9	1.4	3.7	± 0.029	3.26	51.80	± 0.33	2.66
Controls										
No supplement	....	9	6.2	0.9	1.4[2]	±	....	24.48	± 0.56	10.20

[1] Nine determinations.

[2] Six determinations.

the equivalent amount of cod liver oil, but it should be noted that the average daily intake of the irradiated ergosterol lot was 13 per cent less than the average daily intake of the other lots which received this form of the factor. Hence the actual intake was 0.43 unit rather than 0.5. This difference could account for the lower value in the case of this level.

If there were a difference in the response to the two sources of the antirachitic factor, it should have been evident at the 0.5-, 1.0- or 2.0-unit levels, because none of these showed the maximum bone ash percentage which was obtained when 10 units were fed. According to Hume and her associates ('32), 47 to 48 per cent bone ash may be the maximum for a diet low in phosphorus, and Dutcher, Creighton and Rothrock ('25) obtained 52.0 per cent bone ash with the Steenbock 2965 ration plus irradiation, at approximately the same age at which the animals were killed in this experiment. Although higher levels were not fed, in the light of the results of the above investigators, it would seem that 51.18 per cent and 51.80 per cent for the irradiated ergosterol and cod liver oil groups, respectively, at the 10 unit level are maximum values for this ration and age of animal.

Four hundred and fifty milligrams of cod liver oil, 10 rat units, per 50 gm. of ration is equal to 0.9 per cent of the ration. Therefore, the 10-unit level of irradiated ergosterol, which produced the same maximum bone ash response, is equal in its effect on the rat to 0.9 per cent cod liver oil. In the case of the chicken, practically the same percentage of the same sample of cod liver oil, 1 per cent, or 22 rat units per 100 gm. of ration, was four times the amount necessary to produce a maximum bone ash value, but the quantity of the same sample of irradiated ergosterol required to produce a maximum response was 192, or possibly 144 times, the antirachitic value of the cod liver oil at the minimum protective (0.25 per cent) level (unpublished data).

The inorganic P of the whole blood is highest for the groups which received 10 units per 50 gm. of diet and the values for the others became less as the allowance of both forms of the factor decreased. In all instances the phosphorus values of the irradiated ergosterol groups are slightly lower than those of the corresponding cod liver oil groups. It is of interest that the coefficients of variation of the completely protected groups are low as compared with the values for the basal and partially protected groups.

The unit level determined by the usual 10-day curative assay with vitamin D-depleted animals does not afford complete protection when fed on a preventive basis. Schultzer ('31) has compared the preventive and curative methods, but the technics employed are so different from those used in this laboratory that a comparison of results is not possible. At a later date he reported ('33) the preventive dose to be equal to the curative. As indicated above, we have not found this to be the case, but again the methods employed by the two laboratories are so different that comparisons cannot be made.

SUMMARY

1. The response of the white rat to antirachitically equivalent amounts of cod liver oil and irradiated ergosterol, determined curatively, is the same when the two sources of the factor are fed on a preventive basis. This result is strikingly different from that obtained in the chicken, in which species the irradiated ergosterol form of the antirachitic factor is much less effective than the cod liver oil form when compared on a preventive basis.

2. The minimum protective dose on a preventive basis is between 2 and 10 curative units per 50 gm. of ration. The unit or 1+ level, determined curatively, affords only partial protection when fed on a preventive basis.

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STUDIES ON LACTATION

II. TECHNIC FOR STUDYING LACTATION IN RATS

RAY G. DAGGS
Department of Vital Economics, University of Rochester, Rochester, N. Y.

ONE FIGURE

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Most of the existing methods for studying lactation quantitatively are based on humans, cows, goats or dogs as experimental subjects. These methods are time consuming and costly when used in the study of the effects of isolated food substances upon lactation. However, they do afford the opportunity of making quantitative analyses of the milk. One must rely upon the growth rate of the suckling young or some other such factor when small animals like the rat are employed. Methods of this type have the advantages of being relatively rapid and less costly, even though the accuracy is not so great due to the use of indirect criteria of milk production. This inaccuracy can be lessened considerably by carefully controlled experimental conditions and the employment of good growth measurement methods. Methods heretofore used involved mortality and weaning statistics or percentage weight changes of the litter.

In the earlier work on lactation in small animals it was justifiable to use mortality statistics, percentage of young weaned, etc., but since it has become possible to rear rats on a practically synthetic diet, these criteria do not permit the finer discriminations between the effects of various food extracts and concentrates. If one young died in a litter of ten, the per cent weaned would be 90; but if one died in a litter of two the per cent weaned would be 50. The average weaning weight and the per cent increase in weight over the suckling period are of little value, since they depend too largely upon the birth weight which in turn depends to some extent upon the number in the litter. The following table illustrates some of these points.

TABLE 1

NUMBER BORN	NUMBER WEANED	BIRTH WEIGHT OF SIX	WEANING WEIGHT OF SIX	PER CENT WEIGHT INCREASE
7	6	32	197	515
8	6	29	197	579

It is of primary importance to limit the litters to a definite number at birth. Some investigators limit the litters to four young, but most prefer six. Donaldson ('25) has pointed out that rats in relatively small litters grow much faster than rats in larger litters. Another means of lessening the inaccuracy of small animal methods is to disregard the growth data of the first 3 or 4 days of life. There is considerable variation of growth during this period, due to the dehydration of the young and the stabilization of the habits of the mother. It seems advisable, therefore, not only to limit the litters to six young, but to start plotting the growth data from the fourth instead of the first day of life.

Female rats vary somewhat in their inherent ability to mother a litter. This makes it necessary to exclude from the final data all but those experiments where the mother has successfully cared for and weaned the six young allotted to her at birth. It must be remembered that we are here dealing with a method to be used in the study of the augmentation of normal lactation with various food stuffs. We advise including only 'perfect' experiments in the final data, i.e., experiments where the mother has shown no signs of ill health and has successfully cared for all her young remaining after suitable reduction of the litter at birth.

Sugiura and Benedict ('18) have pointed out that a diet may be adequate for reproduction and growth of the young after their eyes have opened, but not for the lactation period. This, then, limits the true lactation period of the rat to 16 or 17 days, since at this time the young open their eyes and begin nibbling at the mother's food. Many investigators have endeavored to prolong this true lactation period (the period over which the young are solely dependent upon the mother's milk for substance). They have devised ingenious food traps, etc. These have all proved unsuccessful, because of the manner in which a rat eats and because of the agility of the young at this age. Kozlowska, McCay and Maynard ('32) have even gone so far as to remove the mother from the cage at definite intervals for separate feedings. This, they admit, is rather impractical and too laborious. We feel that if the conditions are well controlled and only 'perfect' experiments are used in compiling the data, the growth periods from the fourth to the seventeenth day are of sufficient length to make comparisons.

Brody ('27) has analyzed Donaldson's growth data on white rats and finds that growth in the rat is definitely logarithmic and that the instantaneous percentage rate of growth remains constant for relatively long periods of growth. The rate is about 12 per cent for the first 10 days and about 4 per cent for the next 20 days. We have applied essentially his method to our data. Instead of using natural logarithms, we have used ordinary logarithms. The logs of the daily weights of the litters (six young are weighed together) from the fourth to the seventeenth day of life are plotted against time in days. Straight lines are then drawn through the greatest number of points. For this growth period (fourth to seventeenth day) there are two straight lines. The break occurs in the great majority at the tenth day. It may occur a day earlier or a day later. The majority of points fall on the two straight lines. Many other means of plotting and drawing lines were tried, but this method seemed to satisfy the conditions best. A numerical constant is obtained for the slope of each straight line by dividing the log difference between any two points on the line by the distance between them in days. Both of these constants differ with a fair degree of significance when the diets of the lactating mothers differ. Figure 1 shows a compilation of the results obtained on a few different diets that are listed in table 2. The salt mixture used is shown in table 3. It is a slight modification of that used by Clayton ('30).

It is not within the scope of this paper to speculate upon the reason for the sudden change in the rate of growth of the white rat at approximately the tenth day of life. Suffice to say that from a complete analysis of our data we find it occurring in about 90 per cent of the 300 litters studied. Brody gives no conclusive reason for it. It is our belief that it is characteristic of the species, although nutritional conditions may modify it.

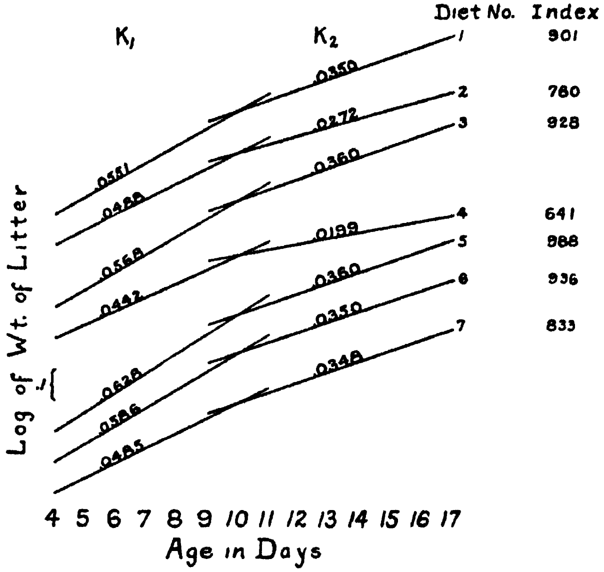

Figure 1

In order to facilitate a quicker comparison of results, we have arbitrarily combined the two growth slope constants by simple addition and dropping the decimal point. The constants thus lose their actual mathematical significance and become merely round numbers to be used for comparison. We have chosen to call the resultant figure a lactation index.

We believe this method is a more accurate picture of the growth changes in the young due to lactation changes in the mother than is presented by any other existing means of judging lactation in rats. Its application and significance will be stressed in the following paper.

TABLE 2

Diets

	PARTS						
	1	2	3	4	5	6	7
Casein		15	25	15	12	15	Stock diet Purina dog chow
Cystine					1	1	
Glycine					1		
Glutamic acid					1		
Cod liver oil				14			
Liver (dried)	22						
Salt mixture	5	5	5	5	5	5	
Agar agar	2	2	2	2	2	2	
Starch	76	76	66	76	76	76	
Lard	14	14	14		14	14	

Wheat germ oil, 5 drops per rat per day on all diets.
Cod liver oil, 6 drops per rat per day on all diets.
Irradiated yeast, 3 gm. per rat per day on all diets.

TABLE 3

Osborne-Mendel salt mixture as modified

	gm.
$CaCO_3$	539.2
$MgCO_3$	96.8
Na_2CO_3	136.8
K_2CO_3 1½ H_2O	675.6
85% H_3PO_4	284.0
Conc. HCl	504.0
Conc. H_2SO_4	21.0
Citric acid (dry)	406.0
Iron citrate	25.36
KI	1.4
$MnSO_4$ H_2O	8.7
NaF	5.0
Alum	4.2
Na_2SiO_3 H_2O	25.0

SUMMARY

A method is described for the study of lactation in rats. It is dependent upon two growth constants derived from plotting the logs of the litter weights from the fourth to the seventeenth day of life against time in days. By combining the two constants a mathematical lactation index is obtained.

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STUDIES ON LACTATION

III. EFFECT OF VARIOUS DIETARY PRINCIPLES ON LACTATION IN RATS

R. G. DAGGS AND R. L. TOMBOULIAN
Department of Vital Economics, University of Rochester, Rochester, N. Y.

ONE FIGURE

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In these studies we are not primarily interested in mammary development, the initial stimulation of milk flow, or the transitory effects of drugs, vasodilators, etc., but rather in increasing the level of milk production already established and still more specifically in why adequate good quality protein maintains a high level of milk production.

Eckles ('16) stated that

> the error is often made of assuming that the richness of the milk varies with the feed. While it is possible under certain conditions to make a variation of possibly 0.2 to 0.4 per cent in the fat by giving certain foods, it is only under conditions so abnormal that it is of scientific interest only and has no practical bearing . . . . The richness of a cow's milk is fixed by heredity and cannot be permanently changed by any means.

It is quite true that the heredity factor, or the individual characteristic of the gland is the most important factor in milk production, but since Eckles made the above statement several workers have succeeded in materially altering lactation by dietary means, especially in rats. The most striking influence has been brought about by feeding a high protein diet. Class A animal proteins have been most successful in stimulating lactation in humans, dogs and rats (Hoobler, '17; Adair, '25; Hitchcock, '26; Daggs, '31, and many others). Of these liver seems to be the outstanding one. If the dietary protein level is too high, kidney hypertrophy and some kidney damage may result. Maynard and Bender ('27, '28) believe that, even though high protein diets do produce enlargements and some degeneration of the kidney in rats, it has no ill effects upon the good health of the animal or its ability to lactate normally.

Increasing the fat or carbohydrate in the diet above a certain optimal level has little if any effect. Meigs ('22), in reviewing this subject, states that

> some authors find that an increase of fat in the food is followed both by an increase milk yield and by an increased concentration of milk fat; others get neither of these results, still others get one without the other . . . . Where moderate amounts of carbohydrate are added to or subtracted from the ration, little or no change follows in either the composition or the quantity of milk.

It is of considerable importance to have an abundance of vitamins in the lactation diet, especially the B complex. The diet must include from three to five times the amount of vitamin B complex necessary for ordinary growth (Macy, Outhouse, Long and Graham, '27). Hartwell ('24) believes this increase is particularly needed when the protein level of the lactation diet is high. There is some disagreement as to which of the B factors is essential. Sure and Walker ('31) believe that vitamin G is not so essential as vitamin B, while Husseman and Hetler ('31) state that vitamin G is more important for lactation than vitamin B, although both are needed. Evans and Burr ('28) have shown that lactating rats need vitamin E in order to prevent muscle paralysis of the suckling young. Vitamin A is also required in fairly large quantities by the lactating mother (Sherman and MacLeod, '25). Sure ('28) was unable to demonstrate the existence of any specific fat-soluble vitamin essential for lactation other than vitamins A and D.

Several investigators have sought a dietary lactagogue among the minerals. Scharrer, Schropp and Schwaibold ('33) have found that the iodine content of the feed increases milk production in cows. Stiner ('27) also has shown this and believes that iodine may enhance milk secretion in women. Since copper has been shown to produce such good results in blood regeneration, Sure ('27) used it as a supplement to vitamin B and found that it was of no value for lactation. The feeding of other minerals in amounts larger than necessary for optimal maintenance has no effect other than changing the mineral content of the milk.

The most promising means of stimulating lactation seems to be linked in some way with proteins. In work with dogs (Daggs, '31), it was shown that liver was a very good source of the lactation stimulating substance. Wilkinson and Nelson ('31) find a lactation promoting substance in fresh liver that is destroyed at 120°C. and is not soluble in ether. Smith and Seegers ('34) have found an alcohol and water soluble, ether insoluble, substance from liver to be effective in stimulating lactation in rats. They believe the lactagogue is not a protein, but is associated with it.

Mapson ('32) believes a water extract of autolyzed liver, which he names 'physin,' to be a lactagogue. He also says it is not a protein, but is associated with proteins and is probably of a hormone nature. It is his belief that 'physin' acts by stimulating the anterior pituitary. Jones and Gersdorff ('34) criticise Mapson and others for using Glaxo casein, which they have shown to be low in cystine and suggest that "cystine should be given serious consideration as a contributing factor."

EXPERIMENTAL

Wistar strain rats were used in the following experiments. The animals were kept in a room where the temperature was controlled to 82°F. ± 1°. The humidity was kept at about 40 per cent and the room was ventilated. The stock and breeding colony was maintained on a ration of Purina dog chow, distilled water and extra vitamin A once a week in the form of Patch's cod liver oil. By means of palpations, the pregnant females were selected for experimental diets about

4 to 7 days before parturition. The pregnant rats were placed in special cages.

Rat cages. Galvanized iron trays with 4-inch high sides were made just to fit into the ordinary square wire cages. The high sides protected the young from drafts and outside disturbances. The mothers were provided with nesting material in the form of clean thin wood chips. Water was provided in Rochester type drinking tubes. The food was placed in small wide mouth glass jars supported in tin cups. The accessory food substances, such as cod liver oil, wheat germ oil, yeast, etc., were placed in glass salt cellars supported in a small galvanized iron rack attached to the front wall of the large tray.

TABLE 1

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Beef liver (dried)	0.0551	0.0350	901	9
Egg (dried) (no lard)	0.0511	0.0391	902	8
Egg (dried) (no lard)	0.0503	0.0363	866	6
Beef round (dried)	0.0511	0.0376	887	7
Beef kidney (dried)	0.0519	0.0374	893	13
Stock (Purina chow)	0.0485	0.0348	833	14

The females remained on the experimental diet until the young were weaned. At parturition the litters were limited to six young. The mother and young were weighed daily. The diet was replenished and the daily dietary supplements were placed in clean salt cellars after each weighing. The method used for judging lactation is described in detail in the preceding paper. Table 2 of the preceding paper illustrated the construction of the diets.

The following tables give compilations of the results of the various diets. The diets are all designated by their sources of protein, etc., the other constituents being essentially the same in all diets, unless otherwise indicated. The protein level, with the exception of a few cases was kept at a minimum so as to determine better the qualitative difference between various protein sources. Table 1 shows the qualitative differences between various natural protein sources. The meats were trimmed free of fat, ground, spread in thin layers on metal trays and dried completely by means of a current of warm air. The dried meats were ground to a powder and incorporated in the diet in fifteen part protein equivalents. The egg used was in the form of ground whole hen-egg flakes. The method of plotting the growth data is illustrated in figure 1 of the preceding paper. K_1 indicates the slope of the first straight line on the growth curve, K_2 the second line, and the lactation index is the sum of the two K's disregarding the decimal. It then becomes merely a figure of relative significance only, and a means of quickly comparing the data.

TABLE 2

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Liver (dried)	0.0551	0.0350	901	9
Liver (dried at high temperature)	0.0456	0.0247	703	10
Egg (dried) (no lard)	0.0503	0.0363	866	6
Egg (cooked in double boiler for 3 hours) (no lard)	0.0500	0.0370	870	6
Casein + a water extract of autolyzed liver concentrated to a paste at low temperature	0.0520	0.0375	895	3
Casein + a water extract of autolyzed liver concentrated to dryness at a high temperature	0.0464	0.0183	647	2

In order to determine whether or not the effective principle was associated with the mineral constituents, the ash of egg was added to the casein diet; when this was done the $K_1 = 0.0484$, $K_2 = 0.0273$, and the index = 757. This was practically identical with the casein index of 760, thus showing no improvement. Mapson ('32) has shown also that liver ash was ineffective and that added manganese produced no favorable effects.

Table 2 illustrates the effect of temperature upon the active principle.

The results of the experiments cited in table 2 show that the principle is partially heat labile or liable to oxidation in the absence of water at higher temperatures. Wilkinson and

Nelson ('31) have shown that the factor in liver is destroyed when heated to 120°C.

Table 3 illustrates the reproducibility of results and the validity of the method. It also shows, as several other investigators have shown, the alcohol solubility of at least part of the principle in liver and kidney. This does not seem to hold for egg. It is probably not easily removed by alcohol from egg.

When the alcohol soluble material from liver was freed of fat with ether and taken up in water, it did not augment the casein diet, but did bring the alcohol extracted liver diet to a value practically as good as that of whole dried liver. Why the alcohol water extract did not augment the casein diet is difficult to understand, unless there is more than one lactation principle.

TABLE 3

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Alcohol extracted liver	0.0509	0.0352	861	7
Alcohol extracted liver (2 years later)	0.0520	0.0342	862	9
Alcohol extracted egg	0.0541	0.0353	894	5
Alcohol extracted egg (2 years later)	0.0534	0.0365	899	16
Alcohol extracted kidney	0.0468	0.0364	832	4
Liver (dried)	0.0551	0.0350	901	9
Egg (dried) (no lard)	0.0511	0.0391	902	8
Kidney (dried)	0.0519	0.0374	893	13
Casein (15 parts)	0.0488	0.0272	760	9
Casein + alcohol-water soluble extract of liver	0.0476	0.0289	765	6
Alcohol extracted liver + alcohol-water soluble extract of liver	0.0548	0.0346	894	12

Since no effect was obtained when the alcohol-water extract was added to casein, it seemed advisable to try the various fatty constituents, although Wilkinson and Nelson ('31) had shown that the factor still remained in the residue after ether extraction of liver, and Smith and Seegers ('34) show its absence in liver fat. Table 4 shows some of these experiments.

The only hint of betterment is seen when liver fat was added to fat extracted liver and it is quite conceivable that some of the alcohol-water soluble material could be carried along as a contaminant. The unsaponifiable fraction of the fat, lecithin, extra wheat germ oil, extra cod liver oil, or extra lard definitely hindered lactation, especially in the latter half of the period. Smith and Seegers ('34) also have shown that vitamin E is not the principle involved.

TABLE 4

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein + unsaponifiable fat from alcohol-ether extract of egg	0.0480	0.0268	748	13
Casein + crude lecithin from egg	0.0464	0.0260	724	3
Alcohol extracted liver + crude liver fat (in place of lard in the diet)	0.0545	0.0336	881	9
Alcohol extracted liver	0.0520	0.0342	862	9
Alcohol extracted liver + alcohol-water soluble extract of liver	0.0548	0.0346	894	12
Casein + 20 drops/day wheat germ oil	0.0454	0.0237	691	6
Casein + 28 parts lard	0.0511	0.0216	727	5
Casein + c.l.o. in place of lard in the diet	0.0442	0.0199	641	3

TABLE 5

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein (25 parts)	0.0568	0.0360	928	11
Alcohol extracted egg	0.0534	0.0365	899	16
Alcohol extracted egg (25 parts)	0.0565	0.0358	923	5
Casein + water extract of autolyzed liver	0.0520	0.0375	895	3
Casein + water extract of autolyzed egg	0.0530	0.0321	851	3
Casein + alcohol-water extract of liver	0.0476	0.0289	765	6
Casein + alcohol-water extract of egg	0.0477	0.0272	749	6

These experiments seemed to limit the lactation principle to the protein fraction. This also was brought out when the fifteen part protein level was increased to twenty-five in the case of casein and alcohol extracted egg, as shown in table 5.

The experiments in table 5 confirm the work of Mapson ('32) in that the lactation principle is readily extracted from

TABLE 6

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein (15 parts) + Armour peptone (2 parts)	0.0510	0.0261	771	3
Casein (15 parts) + Witte peptone (2 parts)	0.0550	0.0335	885	3
Casein (15 parts) + blood fibrin (1 part)	0.0532	0.0322	854	3
Casein (15 parts) + lactalbumen (1 part)	0.0549	0.0310	859	4
Casein (15 parts) + glycine (1 part)	0.0494	0.0243	737	3
Casein (15 parts) + cystine (1 part)	0.0586	0.0350	936	6
Casein (15 parts(+ K_2S (½ part)	0.0513	0.0250	718	3
Casein (12 parts) + cystine (1 part) + glycine (1 part) + glutamic acid (1 part)	0.0628	0.0360	988	3

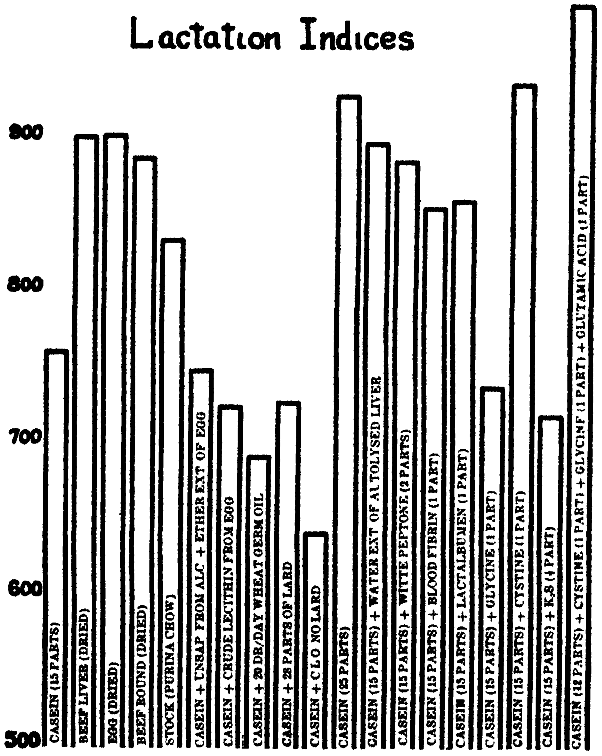

Figure 1

autolyzed liver by means of water. This is also true of autolyzed egg. A larger amount can be extracted in this manner than by alcohol extraction of fresh liver or whole egg.

Since autolysis released a considerable amount of the lactagogue, it was logical to try some of the degradation products of animal proteins.

It will be seen from table 6 that Witte's peptone was better than Armour's. Since we do not know how these peptones are made, we cannot speculate as to the reason for the difference. Blood fibrin, which is the source of some peptones, makes a fair showing. Glycine alone is very poor, but cystine shows a tremendous stimulating effect; in fact, better than anything tried before, even those experiments where 25 parts of liver or egg were used. The effect was evidently not due to added sulphur, since the experiments where K_2S was used gave very poor results. When a synthetic mixture of cystine, glycine and glutamic acid was made to simulate glutathione and fed with the extremely low amount of casein protein, very striking results were obtained. Even if we consider the amino acids sources of protein, the total protein level of the diet is only 15 per cent. It seems, then, that it is not necessary to feed high protein diets in order to maintain a high level of milk production. The high protein diets probably supplied the extra cystine or glutathione constituents necessary for lactation. Figure 1 better illustrates the relative lactation values of some of the diets used.

DISCUSSION

In a short article in Science, Hammett ('34) discusses the function of glutathione. He states,

> Thus, then, it seems as if in glutathione nature has developed in one and the same chemical compound, a complex which conditions if it does not determine the course of the several basic and essential processes concerned in developmental growth. Through cysteine it accelerates cell proliferation, the first step; through glycine it accelerates that protein reconstruction which is an essential accompaniment to both cell division and cellular differentiation; and through glutamic

acid it accelerates the progress of that selective building up of the protein molecule which is the characterizing process of differentiation and its consequent organization.

It may be that cystine or glutathione stimulates the mammary gland as such or is merely passed on to the suckling young through the milk and there acts as a growth stimulant. In order to gain some light on this question, we tried to feed a water suspension of cystine directly to the suckling young of mothers on a basal casein diet. It is very difficult and unsatisfactory to feed cystine to such small beings as suckling rats, but for the few experiments successfully completed the values were $K_1 = 0.0468$, $K_2 = 0.0284$ and a lactation index of 752, slightly below that of a litter from a casein fed mother receiving no cystine. We intend to repeat the experiment on larger animals.

It is definitely known that casein is relatively low in cystine and it may be surmised that the added cystine is merely making a more complete protein of casein. But liver and egg are both considered sources of good complete proteins and even when fed at practically two times the level they did not give as good results as the casein + cystine diet.

The reason why liver is so good for lactation may well be that it contains more glutathione than any other animal tissue. No fact developed in this study stands in the way of this explanation. There is little if any glutathione in fresh hen's egg, but the cystine content is relatively high. Since hot alcohol can 'peptize' proteins, it is probable that the alcohol-water extracts contain cystine-yielding protein products and thus stimulate lactation because of their cystine content. Water soluble proteins from vegetables have been found to contain fair amounts of cystine.

The criticism may arise as to the toxicity of cystine. The percentage is hardly high enough to produce any ill effects. Longwell, Hill and Lewis ('32) have shown that cystine is not toxic when fed with vitamin B complex, and our diets do contain an abundance of B complex.

It is our belief that the lactation augmenting effect of high protein diets, liver extracts, etc., is due to the extra amount of cystine or glutathione contained in the diet and that glutathione particularly acts in some way as a mammary stimulant.

SUMMARY

By using the method described in the preceding paper, lactation studies were carried out on rats using various dietary principles. The food materials tested were either fed in place of the casein of the basal diet or as added daily supplements.

The lactation promoting factor was found to be present in liver, egg, round, kidney, alcohol-water extract of liver, diets of higher protein level (egg casein), water extract of autolyzed liver or egg, Witte's peptone, blood fibrin, lactalbumen, cystine and a combination of 1 part cystine, 1 part glycine and 1 part glutamic acid fed with only 12 parts of casein and the other constituents of the basal diet. This last mixture which simulates glutathione gave the best results, although cystine alone as supplement showed a remarkable effect.

The factor was not present in the unsaponifiable fat of egg, in lecithin, extra wheat germ oil, extra cod liver oil, extra lard, Armour's peptone, glycine or K_2S.

The authors believe that cystine or glutathione acts in some was as a mammary stimulant.

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THE EFFECT OF GRAPE AS COMPARED WITH OTHER FRUIT JUICES ON URINARY ACIDITY AND THE EXCRETION OF ORGANIC ACIDS [1]

RUTH COWAN CLOUSE

Department of Home Economics, The University of Chicago

SIX FIGURES

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INTRODUCTION

Effect of grape juice on urinary acidity. Numerous well-known experiments have clearly established the fact that the ingestion of large quantities of most fruits and vegetables leads, first, to an appreciable decrease in urinary acidity and, second, to an increased output of organic acids. The increased output of organic acids has usually been attributed to the elimination of a certain fraction of the fruit acids which has escaped oxidation in the tissues, although the presence of these acids in the urine has never been proved. Certain fruits, however, notably prunes, plums, and cranberries, have been observed to have an opposite effect, that is, to increase rather than decrease the total acidity of the urine (Blatherwick and Long, '23) and it has been further observed (Slattery, '25) that the ingestion of from 600 to 750 cc. of apple juice (sweet cider) may reduce, rather than increase, the organic acid content of the urine.

That grape juice may also induce a reaction different from that of other fruits is indicated by the conflicting results obtained in certain recent investigations. Pickens and Hetler

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[1] Part of a dissertation submitted to the faculty of the Division of the Biological Sciences of The University of Chicago in candidacy for the degree of doctor of philosophy.

('28) have found that as much as 1 quart of grape juice daily either had no effect upon, or resulted in a slight lowering of the pH of the urine, while Saywell ('32) and Pratt and Swartout ('33 a) have observed that similar quantities of grape juice produced a marked rise in the pH of the urine of their subjects. Pickens and Hetler were, however, working with commercial Concord juice, while the other investigators have used the fresh juice of several varieties of grapes, including the Concord. The difference between his results and those of Pickens and Hetler, Saywell explains on the basis of the probable difference in alkalinity of the fruit juices used, the commercially bottled juice being considerably less alkaline than the fresh, because of the precipitation of tartrates during storage. It is also very probable that different varieties of grapes differ markedly in alkalinity of ash, since the alkalinity of fresh Concord grape juice is reported by Hartmann and Tolman ('18) to be equivalent to 48.6 cc. 0.1 N alkali per 100 cc. of juice, while Saywell reports the alkalinity of the ash of his mixture of fresh Malaga, Muscat and Petit Sirah juices to be equivalent to 74.2 cc. 0.1 N alkali per 100 gm. of juice. In this connection it should be noted that Pratt and Swartout ('33) have observed that Rochelle salts were considerably more effective in reducing the acidity of the urine than was potassium bitartrate—a result which is consistent with the difference in alkalinity of the two compounds.

Relation of organic acid excretion to acid-base balance. It has recently been suggested (Fasold, '30; Schuck, '34 a) that the excretion of organic acids in normal urine may be an important factor in the maintenance of the acid-base balance of the blood of healthy persons. Fasold has observed that the addition of sodium bicarbonate to an already alkaline (vegetable) diet greatly increases the output of organic acids, while the addition of hydrochloric acid to an acid (meat) diet greatly reduces the total organic acid excretion. He has also found ('31) that the administration of sodium or potassium salts of organic acids, such as citric or lactic acids, has the same effect upon the excretion of organic acids as has sodium bicarbonate, while the free organic acids have the same effect as mineral acids, that is, they bring about a reduction in the total organic acids eliminated. The output of any given organic acid, for example, citric or lactic acid, when given by mouth, is sometimes increased, but may actually be reduced in the urine, especially in children. These results have recently been confirmed and extended by Schuck ('34 b) who reports that total organic acid excretion was decreased by the addition of citric acid to a constant, slightly acid basal diet, but was doubled or even tripled by the addition of chemically equivalent amounts of sodium citrate. Citric acid excretion was decreased in some cases and increased in others as the result of the citric acid ingestion, while the sodium citrate brought about a marked increase in every case. Additions of sodium bicarbonate to the basal diet also brought about small increases in the total organic acid excretion and large increases in the citric acid.

Thus, when alkalies are fed the organic acids in general, and citric acid in particular, appear to be important factors in excreting the excess. However, the output of no one acid, even citric acid (Schuck, '34 a), is sufficiently increased to account for the total increase in organic acid excretion. According to Fasold, therefore, "it is possible that there is a group of acids which . . . . through the summation of their small increase in administration of alkali, effect a greatly increased output." Of the organic acids which may function as alkali excretors Fasold believes citric acid to be the most important. Others acids which may play a minor role are lactic and hippuric.

It is very probable, therefore, that the increased organic acid excretion which usually accompanies a fruit diet does not simply represent the excretion of unoxidized portions of the fruit acids, but is one of the mechanisms involved in the adjustment of the acid-base balance. As further evidence for this view, McLaughlin and Blunt's observation ('23) of an increased excretion of uric acid after a fruit meal may be cited.

EXPERIMENTAL

The following investigation was undertaken for the purpose of studying the effect of grape juice as compared with the juice of other fruits and vegetables on the acidity and total organic acid content of the urine. A second aim was to determine, if possible, the identity of the organic acids excreted on the fruit juice diets. The investigation was divided into three separate parts which will be described as experiments I, II and III.

Experiment I

Method: General procedure and diet. The first experiment was intended to be preliminary only, but the results are of sufficient interest to warrant their inclusion here. Five normal young women acted as subjects and two different fruit or vegetable juices were tested on each subject. The experiment was divided into four periods of 3 days each, as follows: period 1, basal diet; period 2, basal diet plus 1 pint of a specified fruit juice; period 3, basal diet; period 4, basal diet plus 1 pint of a second fruit juice. One preliminary day for adjustment to the fruit juice diet was usually allowed between the basal and the fruit juice periods. The basal diet was nearly neutral in reaction and the fluid intake was kept constant at about 2 liters.

The fruit and vegetable juices used were commercially bottled grape juice, both Concord and Catawba; freshly pressed juice from Thompson seedless grapes; freshly pressed orange, lemon, grapefruit, and pineapple juices; commercially bottled loganberry juice, canned sauerkraut juice; and canned tomato pulp.

Chemical analyses. The following determinations were made on 24-hour specimens of urine, collected and preserved under toluene: pH, colorimetric; titratable acidity, according to Folin ('03); and organic acids according to Van Slyke and Palmer ('20).

Results. A summary of the results of this experiment, together with the estimated alkalinity of the ash of certain of the fruit juices used, is given in table 1. All of the juices tested were effective in decreasing the acidity of the urine, although all were not equally so. In general, the rise in the pH of the urine appeared to parallel the increase in alkalinity of the ash of the fruit juice used. Of the three varieties of grape juice tested, the fresh Thompson seedless juice was most effective in reducing urinary acidity, while the bottled Concord juice was least so. It seems highly probable that this result reflects a difference in the alkalinity of the ash of the three varieties of grapes. A further, very interesting and suggestive result of this experiment was that, with the exception of the Concord grape juice, the greatest increase in organic acid excretion occurred on the diets containing the most highly alkaline fruit juices.

TABLE 1

Average increase in urinary pH and total organic acid excretion of five normal women on diets containing 1 pint of a specified fruit or vegetable juice, together with the total alkalinity of the ash of some of the fruit juices used[1]

FRUIT JUICE	URINE		FRUIT JUICES
	Average increase in pH	Excess organic acid	Excess alkalinity of ash per 100 gm. juice[2]
		per cent of intake	*cc. N*
Lemon	0.4	8.1	4.55
Grape, Concord (bottled)	0.5	12.5	1.94
Grape, Catawba (bottled)	0.6	7.0	....
Grapefruit	0.6	5.9	4.08
Grape, Thompson seedless (fresh)	0.7	7.3	....
Loganberry	0.7	1.9	....
Sauerkraut	0.7	7.5	....
Orange	0.8	11.8	5.70
Pineapple	0.8	10.7	7.20
Tomato	1.0	11.4	7.19

[1] Figures for Catawba and Thompson seedless grapes, and for loganberry and sauerkraut juices are omitted in the table because average figures for these juices were not found.

[2] Estimated from Sherman ('27) and Sherman and Gettler ('12).

Experiment II

The second experiment was planned to make a further study of the effects of grape as compared with orange and apple juices upon urinary acidity and also upon the calcium and phosphorus retention of adults.[2]

Orange juice was selected because of the widespread popularity of this fruit juice and because of the reports of other investigators as to its effectiveness in reducing urinary acidity (Blatherwick and Long, '22; Chaney and Blunt, '25) and in promoting calcium and phosphorus retention in children (Chaney and Blunt, '25). The apple juice was chosen because it seemed desirable to investigate further the decreased organic acid excretion found by Slattery ('25).

Method: General procedure. Since calcium and phosphorus retentions were to be determined, a complete metabolism study was carried out on each subject. A low-calcium weighed diet was taken, and both urine and feces, together with aliquot portions of the food, were collected and preserved according to the accepted technic for such studies.

Three normal women served as subjects. Since it seemed desirable to study the effects of all three juices in each of the three subjects, the experiment was divided into six periods, as follows: period 1, basal diet; period 2, basal diet plus 525 gm. bottled apple juice (commercial sweet cider); period 3, basal diet; period 4, basal diet plus 500 gm. freshly pressed orange juice; period 5, basal diet; period 6, basal diet plus 500 gm. bottled Concord grape juice. A preliminary period of 3 days on the basal diet, without collection of urine or feces, preceded period 1. The other periods were separated by one preliminary day in which the fruit juice was fed, but urine collections were not made.

Diet. Computation of the acid-base content of the various diets showed that the basal diet was moderately acid (table 2). The apple and grape juice diets were also slightly acid, but less so than the basal diet, while the orange juice diet was distinctly alkaline. Using Hartmann and Hillig's ('30) figures for the tartaric and citric acid content of grapes and oranges, respectively, and the figures furnished by Slattery ('25) for the malic acid content of apple juice, the intake of these acids in the quantities of fruit juice ingested was computed to be 2.1 to 3.9 gm. malic acid in the apple juice, 4.3 gm. citric acid in the orange juice, and 3.8 gm. tartaric acid in the grape juice.

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[2] The data on calcium and phosphorus retention will be reported in a later paper.

Chemical analyses. The following determinations were made on the 24-hour specimens of urine: pH, colorimetric, according to the method of Hastings, Sendroy and Robson ('25) with the use of the Hastings bicolorimeter; titratable acidity, Folin; total organic acids, Van Slyke and Palmer ('20); and ammonia, Folin and Bell ('17).

TABLE 2

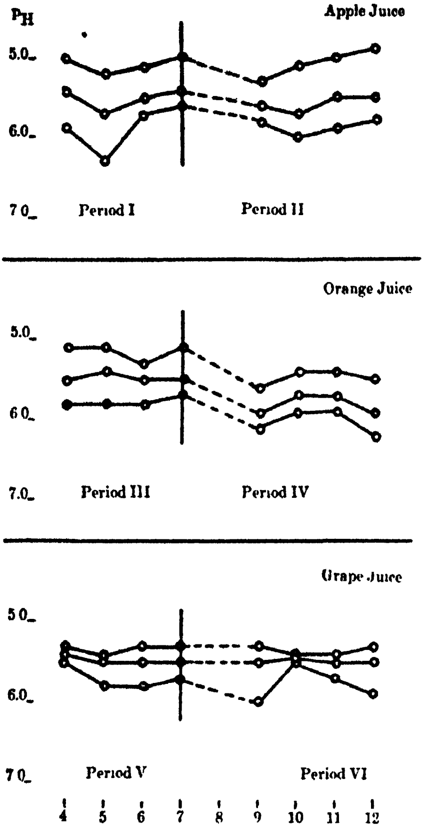
Acid-base content of diets used in experiment II[1]

DIET	PERIOD	EXCESS IN RESIDUE	
		Acid	Base
		cc. N	*cc. N*
Basal	1, 3, 5	19.5–23.4	
Basal, plus 525 gm. apple juice	2	5.8–10.1	
Basal, plus 500 gm. orange juice	4		4.7–8.5
Basal, plus 500 gm. grape juice	6	9.8–13.4	

[1] Estimated from Sherman ('27).

Results. In this experiment apple juice proved to be strongly laxative for two of the subjects, but not at all so for the third. The other two fruit juices were mildly laxative for one subject, but not so for the others. The catharsis appears to have had little consistent effect upon the acidity of the urine, however.

The difference in the effects of the three fruit juices is clearly shown in figures 1 and 2. Figure 1 indicates that, in general, the pH of the urine was raised when approximately ½ liter of orange juice was ingested, but remained the same when similar quantities of apple or grape juice were taken. Figure 2 shows that the total acid excretion (titratable acid



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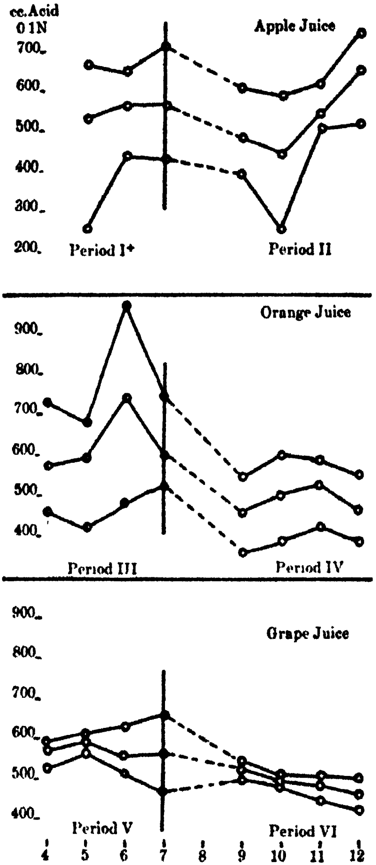

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Fig. 1 Maximum, minimum and average pH of urine of three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). The heavy vertical line indicates the end of the basal diet. The dotted line indicates preliminary period on fruit juice diet.

Fig. 2 Maximum, minimum and average excretion of total acid (titratable acid plus ammonia) by three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet. [1] Ammonia not determined on first test day.

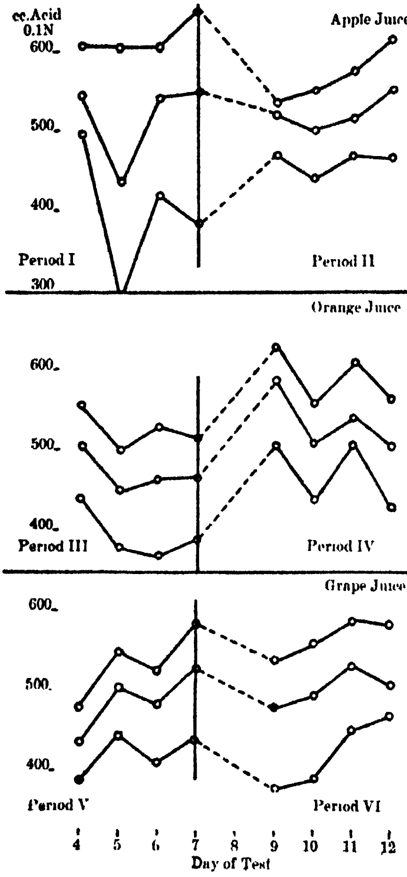
plus ammonia) was reduced on both the grape and the orange juice diets, but that the reduction was somewhat less marked when the grape juice was fed. On the apple juice diet, on the contrary, the total acid excretion tended to increase rather than decrease. A comparison of figures 1 and 2 shows, rather surprisingly, that on the grape juice diet the reduction in total acid excretion was considerably greater than would be indicated by the very slight change in pH.

Organic acids. The excess of total organic acids excreted during the fruit juice periods, expressed in terms of per cent of intake, was greatest during period 4, when orange juice was fed. On the apple juice diet the organic acid excretion was actually reduced in two subjects, although an increase amounting to 6.4 per cent of the intake was observed in the third. In figure 3 are plotted the maximum, minimum, and average excretion of total organic acids in all six experimental periods. A comparison of figures 2 and 3 indicates that the organic acid excretion tended to be the reverse of the total acid excretion; that is, as the total acid output was reduced the organic acid output tended to rise. When there was little change in the total acid excretion, as on the apple juice diets in this experiment, the organic acid output tended to remain practically unchanged.

The CO_2-combining power of the plasma. Fitz and Van Slyke ('17) have found that the total acid excretion (titratable acid plus ammonia) bears a fairly close relationship to the CO_2-combining power of the blood, and that the latter may be computed roughly, with an error of from 3.2 to 7 volumes per cent when the following formula is used:

$$\text{Plasma } CO_2 \text{ capacity} = 80 - \sqrt{D/W}\sqrt{C}$$

where 80 is the maximum CO_2-combining power of the plasma, D the rate of excretion of 0.1 N acid plus ammonia per 24 hours, W the weight of the individual and C the cc. of 0.1 N acid plus ammonia per liter of urine. Recently, Bischoff, Sansum, Long and Dewar ('34) have reported that they could find no correlation between the plasma bicarbonate and either the pH or the titratable acidity of the urine and, further, that any changes which did occur in the plasma bicarbonate as a result of feeding substances having either an acid or an alkaline residue were well within the range of error reported by Fitz and Van Slyke, and also within the range of variation



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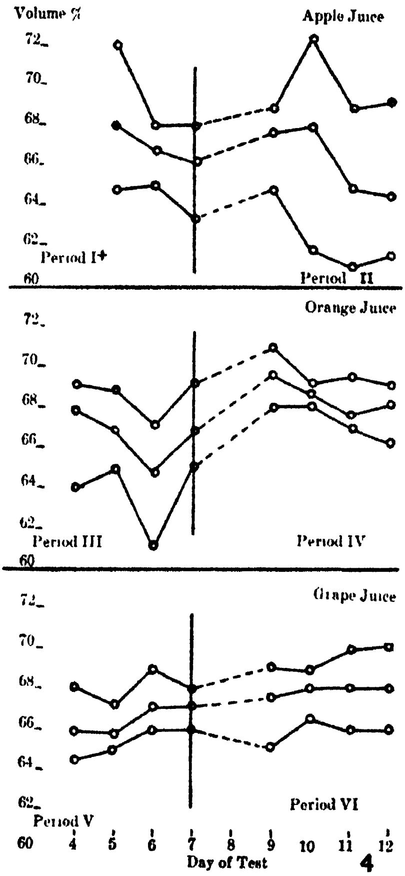

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Fig. 3 Maximum, minimum and average excretion of total organic acids by three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

Fig. 4 Maximum, minimum and average CO_2 binding power of the plasma of three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice. + Ammonia not determined on first test day.

found in the fasting blood (taken before breakfast) of subjects on an ordinary diet. It has, nevertheless, seemed worth while to compute the values for the CO_2-combining power of the plasma for the subjects of this experiment according to the Fitz and Van Slyke formula. The results of these computations are presented in figure 4, which shows an apparent tendency of the blood to become somewhat more acid on the apple juice diet and somewhat more alkaline on both the orange and the grape juice diets. It should be observed, however, that all of the values found in this experiment are within the normal range reported by Fitz and Van Slyke, and also, for the most part, within the range of error reported for the computation. It is therefore quite possible that no significant changes occurred in the blood, although the fact that the different juices appear to have caused different reactions seems of interest.

Experiment III

Method: General procedure. Experiment III was essentially a repetition of experiment II, except that the quantity of fruit juice taken was increased to 1000 gm. daily. Two fruit juices only, orange and grape (commercially bottled Concord), were included and but two subjects were used in the study of each fruit juice.

Diet. The basal diet used in this experiment had a potential acidity of 4.9 to 5.3 cc. of normal acid daily. Both of the fruit juice diets were potentially alkaline, the grape juice diet having a potential alkalinity of 13.0 to 14.1 cc. of normal alkali per day, while the orange juice diet contained an excess of 50.2 to 51.2 cc. of normal alkali daily. The tartaric acid intake in the grape juice diets, estimated as in experiment II, was probably in the neighborhood of 7.6 gm. daily. Since Hartmann and Hillig ('30) have recently reported that grapes also contain an average of 0.023 per cent of citric acid, the intake of citric acid on the grape juice diet probably amounted to about 0.23 gm. daily. The citric acid in the orange juice, as determined by Schuck ('34 a) amounted to 8.51 gm. daily.

Chemical analyses. pH, titratable acidity, and total organic acids were determined on the 24-hour specimens of urine as in experiment I. Ammonia determinations were not made, but instead the uric acid and citric acid output was determined daily, the former by the Folin-Shaffer method (described by Mathews, '30), and the latter by the method described by McClure ('22).[3]

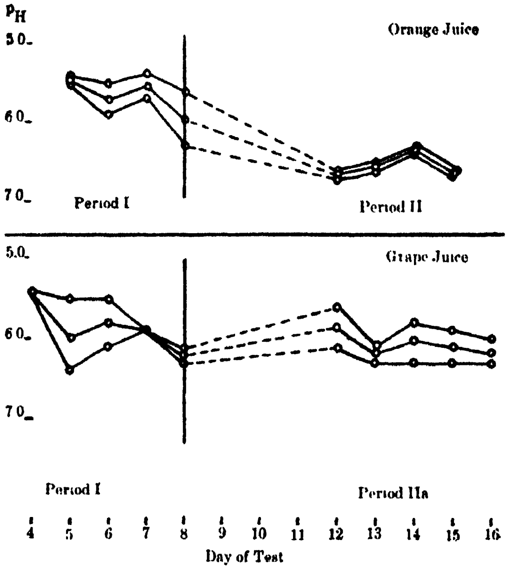

Fig. 5 Maximum, minimum and average pH of urine of two subjects on a basal diet and on the same diet supplemented with 1000 gm. of a specified fruit juice. Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

Results. No laxative effects of either of the fruit juices used were felt by any of the subjects in this experiment.

Urinary acidity and total organic acids. The results of experiment III are presented in figures 5 and 6. Figure 5 shows that the larger quantity of orange juice brought about a very distinct lowering of the acidity of the urine, the effect of 1000 gm. of orange juice being much more marked than that of a similar quantity of grape juice. A comparison of figures 1 and 5 also shows that the larger quantities of juice were decidedly more alkalinizing than the smaller quantities used in experiment II.

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[3] The citric acid determinations were made by Miss Cecilia Shuck as a part of a further study of the metabolism of this acid ('34 a).

Figure 6 shows that in this experiment the increase in the output of total organic acids was quite similar on the two juices, the increase again being greater than that which occurred in experiment II (fig. 3).

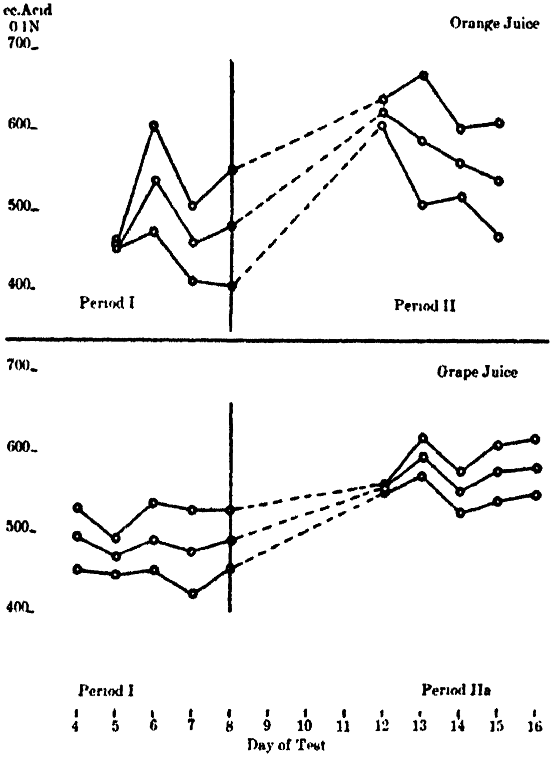

Fig. 6 Maximum, minimum and average excretion of total organic acids by two subjects on a basal diet and on the same diet supplemented with 1000 gm. of a specified fruit juice. Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

Uric and citric acids. Table 3 shows that the output of both uric and citric acids was increased in all subjects on both the grape and orange juice diets. The increased uric acid output, while not large, was sufficient to account for 2.3 to 12.8 per cent of the average increase in total organic acids during the fruit juice periods. On the orange juice diet the increase in citric acid output amounted to an average of 0.234 and 0.457 gm. per day, or 2.8 to 5.4 per cent of the intake. On the grape juice diet the increase in citric acid amounted to the surprisingly high average of 0.148 and 0.275 gm. per day, an increase of 64 per cent of the intake in one subject, and more than 100 per cent in the other. Table 4 shows that the sum of the

TABLE 3

Average total organic, uric and citric acid output of four normal women on a basal diet and on the same diet supplemented with 100 gm. of a specified fruit juice

DIET	SUBJECT	ACID OUTPUT PER 24 HOURS		
		Total organic	Uric	Citric
		Grape juice		
		cc. 0.1 N	*cc. 0.1 N*	*cc. 0.1 N*
Basal	C	525	0.394	0.871
	H	447	0.363	0.634
Basal plus 1000 gm. grape juice	C	596	0.470	1.019
	H	549	0.382	0.909
		Orange juice		
Basal	R	512	0.341	0.658
	K	442	0.441	0.452
Basal plus 1000 gm. orange juice	R	625	0.422	1.115
	K	537	0.479	0.686

TABLE 4

Comparison of the sum of the excess uric plus citric acids excreted on diets containing 1000 gm. of a specified fruit juice with the excess of total organic acids excreted on the same diet

FRUIT JUICE	SUBJECT	EXCESS ACID						
		Total organic	Uric	Citric	Uric plus citric		Undetermined	
		cc. 0.1N	*cc. 0.1N*	*cc. 0.1N*	*cc. 0.1N*	*per cent*	*cc. 0.1N*	*per cent*
Orange	K	94.0	4.5	36.5	41.0	44	53.0	56
Orange	R	113.0	9.6	71.4	81.0	72	32.0	28
Grape	C	70.2	9.0	23.1	32.1	36	48.1	64
Grape	H	100.4	2.3	41.7	44.0	44	56.4	56

excess uric plus citric acids in the urine during the fruit juice periods represents 44 and 72 per cent of the excess organic acid on the orange juice diet, and 36 and 44 per cent of the excess acid output on the grape juice diet. This leaves approximately one-quarter to one-half of the excess organic acid unaccounted for on the orange juice, and about one-half to two-thirds unaccounted for on the grape juice diets.

It is regrettable that determinations of tartaric acid output could not have been made in this study. However, the work of other investigators throws some light on this point. Pratt and Swartout ('33 b) were unable to find tartaric acid in the urine of their subjects after the ingestion of 1000 gm. grape juice, although they say that they "could not devise a practical procedure that would detect tartrates in a solution more dilute than one part in two thousand." Finkle ('33) has observed that 85 to 98.5 per cent of pure neutral sodium tartrate injected intramuscularly appeared in the urine within a few hours, but both Finkle and Underhill and his associates ('31) report that when from 1 to 10 gm. of either tartaric acid or its salts are taken by mouth a maximum of 20 per cent of the quantity ingested appears in the urine. No tartaric acid has been found in the feces in the investigations quoted, but the decomposition of the tartrates by human intestinal contents and by the common fecal bacteria has been observed by Pratt and Swartout ('33 b) and by Underhill and his associates ('31). It is concluded by the latter investigators that a small portion only of the tartrate taken by mouth is absorbed by man and that the human body has no power of oxidation of tartaric acid.

In view of the above facts, it is possible, though it hardly seems probable, that the entire amount of undetermined excess acid in the urine on the grape juice diet was tartaric. If such had been the case, the total excretion of tartaric acid would have amounted to something less than 5 per cent of the intake. In any event, it is not possible to explain the failure of grape juice to lower the pH of the urine as efficiently as does orange juice on the basis of the excretion of large quantities of tartaric acid.

SUMMARY

The effect of various fruit and vegetable juices, especially of bottled apple juice, fresh orange juice, and bottled Concord grape juice, upon the urinary acidity and total organic acid output of normal adult women has been compared, with the following results:

1. The ingestion of orange juice has produced a more marked effect upon the alkalinity of the urine (as measured by an increase in pH and a reduction in titratable acidity and ammonia) than has the ingestion of either grape or apple juice.
2. The effect of bottled Concord grape juice has been variable, although usually in the direction of an alkalinization of the urine.
3. The effect of apple juice has also been variable, with a tendency toward an increased acidity of the urine.
4. Both grape and orange juices tend to produce an increase in the CO_2-combining power of the blood, as calculated by the Fitz and Van Slyke formula. Apple juice, on the contrary, tends to produce a decrease in CO_2-combining power.
5. The ingestion of both grape and orange juices produces an increased excretion of organic acids. The ingestion of apple juice, however, is often accompanied by a decreased organic acid output.
6. The increase in organic acid output observed on both the orange and grape juice diets is in part due to an increased excretion of uric plus citric acids. The increase in organic acid output is not entirely due, therefore, to the excretion of unoxidized remnants of individual fruit acids.
7. The data on organic acid excretion obtained in this investigation are believed to support the suggestion that the organic acids play a significant role in the maintenance of acid-base balance in the body.

Acknowledgment is gratefully made to Dr. Katharine Blunt for the suggestion of this problem; to Dr. Lydia Roberts and other members of the Department of Home Economics and Household Administration for their helpful interest and advice during the progress of the work; to Mrs. Vida Wentz for technical assistance in the determination of ammonia in the second experiment; and to the students who willingly served as subjects in the various experiments.

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EXPERIMENTAL STUDIES ON THE HYPOPHYSIS CEREBRI

IV. THE EFFECT OF VITAMIN E DEFICIENCY IN THE FEMALE ALBINO RAT

SAM I. STEIN

Department of Anatomy, University of Minnesota, Minneapolis

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INTRODUCTION

Vitamin E deficiency seems to operate differently from general inanition and various other combined inanition factors in bringing about sterility, especially in the female. Since it had been proved that the hypophysis was closely related to reproduction (Smith, '26; Smith and Engle, '27; Ascheim and Zondek, '27; Evans and Simpson, '28), investigators sought to determine in what manner the hypophysis affected or was affected by this type of sterility.

Evans, Burr and Althausen ('27) found on feeding various tissues to animals deficient in vitamin E that the hypophysis was slightly richer in that vitamin than any other gland. Vérzar ('29) showed that an abundance of vitamin E has a great stimulating action on the sexual cycle. Preparations containing vitamin E, administered either by mouth or subcutaneously, induced precocious sexual maturity in young female rats, acting similar to anterior lobe hormone. Vitamin E also required the presence of the ovaries for its activity, indicating that it is not directly a building stone of the ovarian hormone, but rather a stimulating or regulating substance for the ovary, as is also the secretion of the anterior lobe of the hypophysis. Vérzar suggested that vitamin E may even be necessary for the building up of the latter.

The earliest anatomical report regarding the effect of vitamin E deficiency on the hypophysis was made by Van Wagenen ('25), who found that in the male rat the hypophysis resembles that of a gonadectomized animal Similar findings for the male have been reported by Nelson ('32, '33) and Mason ('33).

The female rat hypophysis is apparently not affected by a deficiency in vitamin E, according to Stein ('31, a preliminary report), Nelson ('32, '33) and Mason ('33). Only Biceglie ('28) claims to have found a scarcity of acidophil and basophil cells in E-deficient female rats. He attributes these changes to profound degeneration of the ovaries, which also is an observation that none of the other authors have reported. In order to check the above findings more carefully, data derived from ninety-one female albino rats have been worked up statistically and summarized in this paper.

MATERIAL AND METHODS

A. Material

The hypophyses used in this investigation were derived from ninety-one female albino rats (Mus norvegicus albinus) of standard vigorous stock carefully reared and kept from birth to time of autopsy. These animals were used by Dr. J. A. Urner in his studies of "Changes in the uterus and mammary glands of white rats deficient in vitamin E." The following account taken from his outline indicates how the animals were reared.

The low vitamin E diet used in this investigation was developed and furnished by Dr. G. O. Burr, and consisted of casein 60, sucrose 90, salts 9 and lard 40. In addition each animal was individually fed 0.6 gram of powdered yeast and three drops of cod liver oil. This diet was selected because it was found by Evans and Burr ('25) that similar diets, high in sugar, fulfilled all the requirements necessary for growth, but gave no first litter fertility.

The animals were mated on or about the seventy-fifth day of life. The finding of sperm in a vaginal smear, taken twelve hours after the female was put into the male's cage, was considered as evidence that fertilization had taken place and gestation was considered as having begun. When a positive breeding was diagnosed by finding of sperm, a vaginal smear was taken on the fourteenth day for the red blood cell sign. When this was found, the animal was isolated and weighed daily up to the twenty-fifth day, to detect resorbing or normal pregnancy.

The careful rearing and control of this group of pregnant animals, on a low vitamin E diet, from birth through their first pregnancy permitted establishment of a normal for these animals deprived of vitamin E. They were quite normal in appearance in all respects save that the young were absorbed.

After the entire group of animals were carried through this 'sterility' observation on the low vitamin E diet, the animals were devided into two groups. One remained on the diet used throughout the early work, and the other received a 'curative' dose of 0.4 gram of wheat germ daily. Approximately forty animals remained in each group

The second breeding was carried out in the same manner as the first and when a positive breeding was diagnosed, the animal was scheduled for killing upon some particular day of her pregnancy. The cured animals were given their cured ration, beginning with the date of finding sperm in the vagina.

No attempt was made to select animals for one group or the other, and no attention was paid to the division of sister litter mates.

Beginning with the seventh day of gestation, animals of the 'low E' group and 'cured' controls were killed on each successive day, up to and including the twenty-second day.

B. Methods

The animals were killed by being placed in a closed jar containing chloroform.

The gross weight, body length and tail length were recorded. The material was fixed in 10 per cent neutral formalin.

The sections were cut at 5 μ. A volumetric study of the three lobes, a differential cell count and a histologic survey of the anterior lobe were made.

For the volumetric procedure the method followed was that of Hammer ('14). Every tenth section was mounted and stained with haematoxylin and eosin. The image of each section, magnified 33 diameters, was out-lined on paper of uniform thickness, cut out, and the paper weighed on a chemical balance, from which the percentage of each lobe was calculated.

For the histological study and differential cell count, about five sections at well-distributed intervals were taken, stained with a modified Mallory's triple connective tissue stain so that the nuclei stood out clearly, and made the cell count more reliable.

Three types of cells were distinguished similar to most classifications, namely, eosinophils, basophils and neutrophils.

About 8000 cells were counted in each gland.

No study of pars tuberalis was made for it had been left attached to the brain during autopsy procedure.

For further details regarding technical methods and for statistical formulae, the reader is referred to previous reports of the author's (Stein, '33 a, '33 b).

I want to take this opportunity to thank my advisers, Dr. A. T. Rasmussen, and Dr. C. M. Jackson, for the valuable information they supplied during the course of the work, and Dr. J. C. Urner for permitting me to use the material which made this study possible.

RESULTS

A. Age, body weight and body length

A difference of 17 days exists between the average age of the two groups of animals. This is a possible significant difference (table 1), but probably does not have any effect on the results, for organ weights are much more closely related to body length or body weight (Freudenberger, '32; Donaldson, '24).

No significant difference is found in body weight (table 2) or body length (table 3).

B. Weight of the whole hypophysis

Since pregnancy does not cause any change in the whole weight of the hypophysis in the rat (Stein, '33 b, '34), any change that had occurred in either of these two groups would undoubtedly be due to the deficiency in vitamin E. However, no significant difference was found in the average weight of the whole gland of these animals (table 4).

TABLE 1

Statistical constants for age of animal, days

ANIMALS	NO.	M ± S.E.m[1]	Diff. ± S.E.Diff[1]	Diff. S.E.Diff.[1]	P[1]	C.V.[1]
E-deficient	19	136 ± 5.39				17
E-cured	19	153 ± 6.45	17 ± 8.41	2.02	4.3	18.3

[1] M = mean; S.E.m = standard error of mean; Diff. = difference between the mean of the control and the mean of the test; S.E.Diff. = difference divided by the standard error of the difference; P = number of chances in 100 that the differences may be due to errors in random sampling; C.V. = coefficient of variation.

TABLE 2

Statistical constants for body weight, grams

ANIMALS	NO.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	229.6 ± 5.40				10.2
E-cured	19	241 ± 7.03	11.4 ± 8.87	1.29	20.0	12.7

TABLE 3

Statistical constants for body length, centimeters

ANIMALS	NO.	M ± S.E.m	Diff ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	20.3 ± 0.49				10.5
E-cured	19	20.3 ± 0.37	0 ± 0.61	0	0	7.8

TABLE 4

Statistical constants for weight of whole hypophysis, milligrams

ANIMALS	NO.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	12.41 ± 0.48				16.3
E-cured	19	12.8 ± 0.67	0.39 ± 0.82	0.47	63.0	22.8

TABLE 5

Statistical constants for absolute and relative weights of the individual lobes

ANIMALS	M ± S.E.m	Diff. ± S.E.Diff.	$\frac{\text{Diff.}}{\text{S.E.Diff.}}$	P	C.V.	M ± S.E.m	Diff. ± S.E.Diff.	$\frac{\text{Diff.}}{\text{S.E.Diff.}}$	P
	Pars anterior (absolute weight)					Pars anterior (relative weight)			
E-deficient	11.15 ± 1.08				9.6	87.77 ± 0.97			
E-cured	11.21 ± 0.64	0.06 ± 1.26	0.05	96	24.7	87.39 ± 0.57	0.38 ± 1.12	0.34	74
	Pars intermedia (absolute weight)					Pars intermedia (relative weight)			
E-deficient	0.57 ± 0.03				22.0	4.61 ± 0.21			
E-cured	0.60 ± 0.04	0.03 ± 0.05	0.64	53	26.6	4.82 ± 0.24	0.21 ±0.32	0.66	51
	Pars posterior (absolute weight)					Pars posterior (relative weight)			
E-deficient	0.93 ± 0.04				18.7	7.60 ± 0.29			
E-cured	0.96 ± 0.05	0.03 ± 0.07	0.45	65	24.0	7.78 ± 0.40	0.18 ± 0.50	0.36	72

C. Absolute and relative volume of the lobes

It is unnecessary to quote the individual figures listed in table 5 to show the striking similarity which exists between the absolute and relative weights of the various lobes of the two groups. Certainly, there is no evidence that vitamin E affects any of the individual lobes. Furthermore, on comparing the relative weights of the various lobes of these groups with those of normal non-pregnant or pregnant animals (Stein, '33 b, '34), no significant difference is found.

D. Relative number of different types of cells in the anterior lobe

A possible significant difference may be present in the differential cell count (table 6). There are about 2.8 per cent more chromophobes in the cured group, with a corresponding increase of about 2.9 per cent of acidophils in the deficient group, the percentage of basophils having remained equal. Since the significance of the difference between the means was tested by the statistical principle that a difference between two means is possibly significant when it is two times its standard error, and probably significant when it is three times its standard error, the differences noted above are probably not significant, for both of them are only slightly more than two times their standard error (table 6). As was mentioned, the per-

TABLE 6

Statistical constants of percentages for differential count of cells in anterior lobe

ANIMALS	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P
		Chromophobes		
E-deficient	80.77 ± 1.00			
E-cured	83.56 ± 0.73	2.79 ± 1.24	2.25	2.4
		Acidophils		
E-deficient	17.60 ± 1.01			
E-cured	14.72 ± 0.73	−2.88 ± 1.25	2.31	2.1
		Basophils		
E-deficient	1.63 ± 0.10			
E-cured	1.72 ± 0.07	0.09 ± 0.13	0.71	48

centage of basophils showed no significant difference (1.63 per cent for the deficient group and 1.72 per cent for the cured group). Since it is the basophilic cell which alters as a result of a deficiency of vitamin E in the male, the similarity of the above two figures indicated that no such change occurs in the female.

HISTOLOGY

No sign of the typical castration cell could be found in any of the glands, either of the deficient or cured group, such as were found in the vitamin E-deficient male rat hypophyses. This finding for the female is in agreement with that of Nelson ('32) and Mason ('33).

CONCLUSIONS

1. No significant differences were found in the weight of the whole hypophysis or in the absolute and relative volume of the lobes in nineteen female white rats made sterile by a deficiency of vitamin E, as compared with the lobes of the hypophysis from nineteen pregnant white rats cured of vitamin E deficiency.
2. A differential count of the cells in the anterior lobe shows that there is probably no significant difference in the percentage of the cured and non-cured. This is particularly true for the basophilic cells.
3. No evidence of any histological change could be found resulting from the vitamin E deficiency such as has been claimed for the male animal.

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THE VITAMIN E CONTENT OF EGGS AS RELATED TO THE DIET OF THE HEN AND TO HATCHABILITY

GLENN L. BARNUM

Animal Husbandry Division, Bureau of Animal Industry, U. S. Department of Agriculture

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The presence of vitamin E in eggs was established early in the investigations on the occurrence of this factor. Evans and Burr ('27) describe rat feeding experiments which showed that one-third of a whole egg fed daily as a supplement to a vitamin E-deficient diet permitted normal reproduction. However, they failed to obtain litters when 100 mg. of an ether extract of egg yolk when fed daily as a curative dose to female rats suffering from vitamin E-deficiency. Card et al. ('30) have recently studied the performance of pullets raised on a vitamin E-deficient diet. Their experiments indicated that vitamin E was essential to normal reproduction in the fowl. In studying the possible relationships of vitamin E to egg hatchability and the development of the chick, Adamstone ('31) showed that fertile eggs produced by pullets fed on vitamin E-deficient rations failed to hatch a normal proportion of healthy chicks. The embryos from eggs produced on the deficient diets usually died by the end of the fourth day of incubation or shortly thereafter. In discussing embryonic mortality and its probable cause in the chick, Byerly ('30) showed that the early peak in the mortality curve occurred on the third day, and also reported the frequent occurrence of a peak in the curve at about the middle of the incubation period.

The possible destruction or inactivation of vitamin E in the diet is suggested from the work of Mattill ('27), who reported results showing the high sensitivity of vitamin E to the destructive action of rancidity in fats. That the efficacy of a given source of vitamin E depends upon the presence or absence of certain associated fats which function either as pro- or anti-oxidants was further shown by Cummings and Mattill ('31), who attributed the inactivation of the vitamin to the action of peroxides developed during auto-oxidation of fats.

The results presented in this paper on the influence of the diet of the hen on the vitamin E content of the egg were obtained during the progress of studies on the nutritive aspects of egg composition which have been in progress for a number of years. Work has already been reported on some relationships of the diet of the hen to the chemical composition (Titus et al., '33), the hatchability (Byerly et al., '33 a, b), and the vitamins A, B and G contents (Ellis et al., '33) of the eggs. Diets low in vitamin E or especially supplemented in this factor were also fed. Determination of the differences in vitamin E content of eggs produced on different diets as well as studies on the presence and stability of vitamin E in the hen diets were made by means of rat feeding tests.[1]

PLAN OF EXPERIMENT

The vitamin E studies extended over a 3-year period on eggs obtained from three series of poultry feeding experiments run in successive years from 1930 to 1933. Each poultry feeding experiment was started in September and was terminated the following August. Rhode Island Red pullets or yearling hens were used in all the work. The housing and management together with the general plans for compounding the diets of the hens have been described in earlier papers by Titus et al. ('33), Byerly et al. ('33) and Ellis et al. ('33).

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[1] The experimental feeding of the hens was conducted under the direction of Harry W. Titus, while the hatchability studies were made under the supervision of Theodore C. Byerly, of the Office of Poultry Investigations. The investigations on vitamin E described in this paper were carried out in the nutrition laboratory under the supervision of N. R. Ellis, also of this division. The material presented in this paper was taken from the dissertation presented to the School of Hygiene and Public Health of the Johns Hopkins University by the author in partial fulfillment of the requirements for the degree of doctor of science. The interest and advice of Prof. E. V. McCollum in the problem is gratefully acknowledged.

Most of the vitamin E studies have been related to comparative work between a normal hen diet on the one hand and various modifications of a deficient diet on the other. The normal diet (no. 1) consisted of 72.3 per cent of the basal mixture of yellow corn meal, wheat bran, rolled oats and alfalfa leaf meal, 20 per cent of a protein supplement composed of meat meal, fish meal and dried buttermilk, together with minerals and cod liver oil. The five deficient diets studied contained pearled hominy, minerals and cod liver oil, together with protein and vitamin supplements. The protein level was kept at 20 per cent by adjustment of the hominy and meat scrap or buttermilk. Following is the list of diets showing the protein and vitamin supplements:

No. 2	Dry yeast cells (15 per cent) and meat scrap.
No. 3	Rice bran (15 per cent) and meat scrap.
No. 3a	The no. 3 diet treated with ferric sulphate to inactivate the vitamin E.
No. 4	Dried buttermilk.
No. 5	Wheat germ (15 per cent) and meat scrap.
No. 6	Meat scrap (the basal deficient diet) and one head of lettuce daily for every seven birds.

Diets 1 and 3 were used all 3 years and the other diets during a single year. The eggs produced by the birds on these diets were studied both for their hatchability[2] and their vitamin E content. The testing of the eggs produced, as well as the diets fed to the hens for their vitamin E content were carried out on rats according to the general technic used by Evans and Burr ('27) and further studied on male rats by Mason ('29).

Male and female rats, when 4 weeks old, were placed in individual cages, with raised screen bottoms, and fed a basal vitamin E-deficient diet. This diet consisted of: alcohol extracted casein 20 per cent; lard 15 per cent; minerals[3] 4 per cent, agar 1 per cent; dry yeast 8 per cent; and corn dextrin 52 per cent. The cod liver oil was fed in separate glass containers to prevent its coming in contact with the lard in the diet.

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[2] The results of hatchability studies made under the supervision of Dr. Theodore C. Byerly were kindly made available for reference in the present paper in order to show certain relationships of embryonic mortality to the vitamin E content.

The young females were mated for initial pregnancies at the age of 100 days. After observance of the mating plug, the animals were left in individual cages, examined for the implantation sign at the appropriate time, then weighed daily until delivery of litter or if no litter resulted until approximately the twenty-third day. Evidence of resorption rested primarily upon the weight curve. After initial resorption, feeding of the test material was started and the females again mated.

The testing of the poultry diets was done by supplementing the vitamin E-deficient rat diet with daily feedings at various levels of the poultry diet or of the substitution of the poultry diet for the vitamin E-deficient diet. Since rancidity has been shown to be an important factor in the destruction of vitamin E, the poultry diet supplements were fed in separate containers in order to prevent their coming in contact with the basal rat diet.

The eggs for the reproductive studies were collected and stored at approximately 7°C. The eggs were seldom over 2 weeks old when used, and were prepared for feeding by mixing the entire egg and pipetting the desired amount into small dishes. The egg testing was done during the spring and summer months during the period of maximum egg production. Lettuce and wheat germ were also tested both from the point of view of a check on the performance of the rats and on the material used in the hen diets. In the usual case the test material was fed approximately 2 weeks prior to conception and throughout the gestation period. All feedings were strictly comparable even though carried over a longer period than is generally considered necessary for prevention of resorption. The sharpness of the results of the test feeding at definite daily levels attest to their validity. Emphasis was thus placed upon the comparative daily effective dose rather than the total amount of supplemental material. For example, eggs produced from hens on the normal diet fed at a 3-cc. level were only partially effective in preventing resorptions, while the 5-cc. level was totally effective. If a resorption as determined in the usual manner resulted in the second gestation, the female was used in test work again. After the female had undergone three or four resorptions, she was fed sufficient test material to produce a litter, thus proving her reproductive efficiency. After a litter, females were always allowed to mate and undergo resorption to insure depletion before being used for test work again.

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[3] Calcium carbonate 75.1 parts; calcium phosphate (dibasic) 107.6 parts, potassium phosphate (monobasic) 69.8 parts; potassium sulphate 22.0 parts; magnesium carbonate 21.0 parts; potassium carbonate 35.4 parts; sodium chloride 35.0 parts; ferric citrate 6.3 parts; potassium iodide 0.25 parts; manganese sulphate 1.0 parts; sodium fluoride 0.5 parts; potassium aluminum sulphate 0.5 parts; sodium silicate 1.0 parts; and copper sulphate 0.5 parts.

Male rats were also used during the first year's work. Inasmuch as impairment in the male is permanent, the prophylactic type of experiment was used in order to study the preventive effect of the test material. In these tests the male rats were given daily test feedings of eggs or other material to be tested when started on the experiment. They were allowed to remain on this regimen for 119 days and were then studied for the dietary effects on testicular degeneration. In some cases functional tests were made, but, in general, the routine studies were made by; a) an examination of the spermatozoa by clipping the epididymis and noting motility, clumping and aspermia; b) histological study of both right and left testes; c) weight of trimmed testes. The weight of the testes was taken and the percentage of the normal was calculated according to Donaldson ('24).

RESULTS

Lettuce and wheat germ. One group of female rats was fed 5 gm. of fresh head lettuce daily during the entire gestation period. There were seven pregnancies in this series and four of these resulted in litters, while the remaining three terminated in resorptions. Two controls fed lettuce supplements at the rate of 2.5 gm. daily in the prophylactic series produced normal and healthy litters. The lettuce fed in these experiments was the solid type of head lettuce obtained on the local market and no effort was made to select it according to the greenness of the leaves or to separate the leaves from the stock. Evidently this lettuce contained less vitamin E than that used by Evans and Burr ('27), since they found that 2.5 gm. daily doses of fresh green leaves fed therapeutically was effective in preventing resorptions.

Another control group was fed approximately 2.0 gm. of wheat germ daily. Eight pregnancies were studied in this series and each one resulted in a litter. Several rats in this series were allowed to re-mate a few weeks later, after being placed on the deficient ration without supplements of wheat germ or other sources of the reproductive factor. The resulting pregnancies also terminated in normal litters. In addition to the excess in the minimum effective dose indicated in feeding 2 gm. daily, further data are available showing that 1 gm. of wheat germ was sufficient to produce normal litters.

The poultry diets. The two diets most extensively used in the hatchability studies were the normal diet and the deficient rice bran diet. These diets were typical of those fed to pullets and hens which produced eggs of normal and of low hatchabilities. The diets were first tested for vitamin E content without the cod liver oil incorporated. As shown in table 1, little or no difference in their vitamin E content was found, since rats fed 5 gm. daily of either of the two diets were unable to produce litters, while 10 gm. of both diets permitted normal births.

The apparent difference in response of the rat and the hen to these two diets suggested a further examination of the diets with the cod liver oil incorporated into them at the same level and under comparable time and temperature conditions as fed to the hens. Accordingly, the cod liver oil was added at the rate of 2 per cent to the normal diet and the rice bran diet, which were then held in trays to permit free access of air at a temperature of approximately 25°C. for 10 days. These rations were then tested for vitamin E as before. From table 1 it will be seen that the normal diet with cod liver oil added produced three resorptions and seven litters out of ten pregnancies studied. This result indicates that there was some destruction, although not as marked as in the rice bran diet (no. 3) with cod liver oil added where six pregnancies resulted in resorptions and one in a litter. The rice bran oil present in this diet is suggested as a contributing factor in reducing the proportion of resorption cures.

TABLE 1

The vitamin E content of poultry diets as determined by feeding tests with rats

DIET NO.	DAILY DOSE	NUMBER OF FEMALES	PREGNANCIES	RESORPTIONS	LITTERS
	gm.				
1	5	4	4	4	0
(Normal)	10	7	7	1	6
No cod liver oil)	ad lib.	7	7	1	6
3	5	4	4	4	0
(Rice bran)	10	6	6	0	6
No cod liver oil	ad lib.	13	13	1	12
1 (Normal) With cod liver oil	ad lib.	10	10	3	7
3 (Rice bran) With cod liver oil	ad lib.	7	7	6	1
1 (Normal) Iron treated	ad lib.	11	11	1	10
3a Iron treated rice bran diet	ad lib.	5	6	6	0

Further study of the comparative destruction of the two diets was made by treatment with iron salts. The normal diet when so treated showed little loss in vitamin E potency.

The rice bran diet before treatment allowed the birth of normal litters in twelve of the thirteen pregnancies studied on ad libitum feeding, while after treatment resorptions (table 1) resulted in all of the six pregnancies studied.

The results of the tests on the poultry diets, including iron treatment as well as those with and without cod liver oil incorporated, apparently indicate that the rice bran possessed either a less amount of vitamin E or was more susceptible to destruction or inactivation of this factor than the normal diet.

The vitamin E content of the eggs. The eggs prepared as previously described were fed to vitamin E depleted female rats in doses of 3 cc., 5 cc., and 10 cc. daily. The eggs from the normal pen, when fed at 3 cc. levels (table 2) produced litters in five of the fourteen pregnancies during the 3 years' work. This level was apparently a borderline dose, since other rats fed at a level of 5 cc. produced litters in the ten pregnancies studied.

In contrast with the results on normal eggs, the rat tests on the eggs produced on the yeast diet yielded only one litter out of seven pregnancies on the 3-cc. and no litters out of seven pregnancies studied on the 5-cc. level. In the series on the eggs from the pens which received rice bran, little if any difference in vitamin E content was apparent between the diet treated with iron sulphate and the untreated diet. Eggs produced on diet no. 3 yielded no litters out of eight pregnancies on the 10-cc. level. Accordingly, the results were not unusual on diet no. 3a with thirteen resorptions out of fifteen pregnancies on the 5- and 10-cc. levels. In fact, the treatment of the hen diet with the iron salt appeared quite unnecessary after the findings on the no. 3 diet (rice bran).

The deficient eggs produced by the hens on the diet using buttermilk as the source of protein were studied on the 5-cc. daily dose only. Six pregnancies were observed and only one litter resulted. Three cubic centimeters of egg produced by hens fed the wheat germ diet were effective in producing four litters out of the five pregnancies studied as compared to one litter in eight pregnancies with eggs from the pen with rice bran instead of wheat germ. The daily feeding of the 5 cc. of egg from the wheat germ diet resulted in litters in seven out of eight pregnancies as contrasted to two litters in seventeen pregnancies from the rice bran diet.

TABLE 2

The vitamin E content of whole eggs produced on different diets as shown by the curative effect on female rats

EGGS FROM DIET NO.	DAILY DOSE	NUMBER OF FEMALES	PREGNANCIES	RESORPTIONS	LITTERS
	cc.				
1	3	12	14	9	5
(Normal)	5	9	10	0	10
2	3	5	7	6	1
(Yeast)	5	5	7	7	0
3	3	6	10	8	2
(Rice bran)	5	14	18	15	3
	10	7	8	8	0
3a					
(Iron treated	5	7	7	7	0
rice bran diet)	10	7	8	6	2
4					
(Buttermilk)	5	5	6	5	1
5	3	5	6	2	4
(Wheat germ)	5	7	8	1	7
6	3	4	4	3	1
(Lettuce)	5	9	9	9	0
	10	7	7	6	1

The 3-, 5-, and 10-cc. levels of egg produced on the deficient diet supplemented with fresh lettuce were all ineffective in preventing resorptions in the female rats. Judging by the relative potency of lettuce and wheat germ as measured by the rat tests, the two hen diets were by no means comparable, since the diet with wheat germ was considerably more potent than that with lettuce.

The prophylactic tests with male rats. Studies with approximately seventy male rats on the potency of the eggs produced on the deficient diets supplemented with yeast and with rice bran, as well as those from the normal pen were made. The eggs from the normal pen were shown to be definitely more potent in their vitamin E content than those from the deficient pens. The results from the male tests support, in general, the female rat tests and the hatchability results on the eggs from the respective pens. One cubic centimeter of egg from hens on the normal diet was protective against marked degenerative changes, while 1 cc. of egg from hens on the deficient diets was not generally protective. However, protective feeding tests with male rats and their wide variation in degenerative changes as shown by weight of

TABLE 3

The hatchability and first 4-day mortality of the eggs produced on the diets used in the vitamin E studies

EGGS FROM DIET NO.	NUMBER OF EGGS USED	MORTALITY IN FIRST 4 DAYS OF INCUBATION	HATCHABILITY
		per cent	*per cent*
1 (Normal)	2237	7.0	73.0
2 (Yeast)	734	29.0	16.0
3 (Rice bran)	1836	25.0	26.0
3a (Iron treated rice bran)	168	42.0	18.0
4 (Buttermilk	887	22.0	27.0
5 (Wheat germ)	305	8.0	46.0
6 (Lettuce)	144	17.0	22.0

the testes and histological section make tests with male rats less reliable and hence not as accurate a measure as the female series.

The hatchability of the eggs. Table 3 gives the approximate average hatchabilities of fertile eggs taken from the same pens as used in the vitamin E studies. A high mortality was noted in the first 4 days of the incubation period in the eggs from the yeast, rice bran and buttermilk diets, while the wheat germ showed an early mortality little different from the normal diet. The lettuce diet appeared somewhat less effective than the wheat germ, but more effective than any of the other deficient diets in the prevention of the early embryonic mortality.

Only the normal diet produced eggs of a reasonably high hatchability. All the other diets studied showed low hatching percentages ranging from 16 per cent on the yeast to 46 per cent on the wheat germ diet which indicated that a deficiency in some factor or factors persisted in spite of the various supplements added to or substituted in the deficient mixture of hominy and meat meal. The evident relation of vitamin E to the first 4-day mortality of the embryos is indicative of the important role vitamin E undoubtedly played in reducing the number of chickens hatched.

DISCUSSION

It is evident from the data presented that the vitamin E content of eggs may vary through a considerable range. However, the diet of the hen was the determining factor in producing the differences. While eggs produced on a normal diet were partially effective at a 3-cc. and fully effective at a 5-cc. daily level in the curative type of experimental rat test, 10 cc. of egg from deficient diets were insufficient. Thus, the eggs from the normal diet were fully three times as potent in vitamin E as those from deficient diets. In numerous instances, after a female had undergone a resorption on a given level of deficient eggs, she was again placed on the same level of egg from the same pen during the next pregnancy.

Most of these gestations also resulted in resorptions, while other cases on normal eggs showed cures in the first period of feeding. Frequent pregnancy histories of this nature make the contrast in results all the more striking.

The eggs from the normal and the wheat germ diets showed the highest vitamin E content along with the lowest embryonic mortality during the first 4 days of incubation. The per cent hatchability also showed the eggs from these two diets to rank first and second, respectively. However, the rather wide difference in actual values for hatchability as compared to the agreement in results on vitamin E content shown by the rat tests indicate that a factor or factors other than vitamin E were still lacking in the wheat germ diet (no. 5). Further study of the relative ranking of the eggs shows that the yeast (no. 2) and the treated rice bran diets were the poorest in vitamin E and in hatchability. The eggs produced on the rice bran diet (no. 3) appeared to contain little, if any, more vitamin E, although the hatchability was somewhat better than for the iron treated diet. Before incorporation of cod liver oil, the rice bran diet possessed a moderate amount of vitamin E comparable to that of the normal diet. However, rat tests on the complete diet (no. 3) as fed to the hens substantiate the findings on the low vitamin E content and low hatchability of the eggs produced. The inequality between lettuce and wheat germ as it affected the eggs was undoubtedly a question of relative amounts consumed by the hens. In other words, the potency of the head lettuce used was lower than estimated in formulating the diets and the amount supplied did not meet the minimum requirements of the birds. Buttermilk in the diet (no. 4) appeared to be less effective in prevention of the first 4-day mortality, but more effective in improving the hatchability than lettuce. In general, however, the hatchability, the freedom from embryonic mortality early in incubation and the vitamin E content of the eggs rank in approximately the same order.

In studying dietary deficiencies such as these, other possible variables such as vitamins A, B, D and G, as well as possible protein deficiencies and mineral balance have not been overlooked. The fat soluble vitamins A and D were amply supplied in the diets and the experiments of Ellis et al. ('33) have shown adequate vitamin A content of eggs from hens receiving the normal and rice bran diets. These authors further showed that variations in the vitamin B content of the eggs may be produced by diets such as those used in the present studies, but such differences were not directly related to the hatchability of the eggs. The constitution of the diets used in the present studies, however, do not indicate the possibility of vitamin B deficiency. Further experiments of Ellis et al. ('33) do not show pronounced decrease in the vitamin G content of eggs produced on diets very deficient in this factor as compared to the normal diet. The possibility of mineral balance can be ruled out, because the mineral content of all the diets including the normal diet was essentially the same.

The quality of protein in the diets has been suggested by Byerly et al. ('33) as a factor in hatchability in connection with the present studies. However, the use of milk protein in the buttermilk diet effected little change from that of the meat protein in the rat.

From the hatchability standpoint, then, the richness of the diet in vitamin E and the ability of the hen to transmit this factor in an active form into the egg in sufficient amounts becomes a matter of considerable importance. It is true that a somewhat unusual dietary regimen was necessary to produce the differences observed in the present experiments. The requirements of the hen in relation to the supply in ordinary feeds does not appear to be excessive, yet certain combinations of feeds in one way or another may result in the involvement of vitamin E in hatchability. That it is an essential constituent of the diet of the hen appears reasonably certain.

SUMMARY AND CONCLUSIONS

Studies were conducted on the vitamin E content of eggs with relation to the diet of the hen and the hatchabilities of the eggs. By varying the diets in different pens it was found possible to produce eggs varying widely in their hatchabilities. The normal diet was partially effective at a 3-cc. level of eggs and a 5-cc. level was fully effective in curing vitamin E-deficiency in female rats. The eggs from those deficient diets which showed low hatchability were ineffective even at a 10-cc. level. The prophylactic egg tests with male rats yielded results which agreed, in general, with the female rat tests.

The normal diet which produced eggs of moderately high hatchability was found to be potent in vitamin E when tested with female rats. The diets which produced eggs of low hatchability particularly marked by a high first week embryonic mortality were found to be low or lacking in vitamin E, providing cod liver oil was incorporated in the diet of the rats. In the absence of the latter, the rat tests showed little difference in vitamin E content from the normal diet.

When the deficient diet which produced eggs lacking in vitamin E and low in hatchability was supplemented with 15 per cent of wheat germ, the eggs were comparable in vitamin E content to eggs produced on the normal diet. On the other hand, one head of lettuce per seven birds daily did not increase the vitamin E content or markedly improve the hatchability, although it reduced the first 4-day mortality over that of the deficient diet.

It is concluded from the data obtained that vitamin E may be a limiting factor in the hatchability of eggs on certain types of diets and that one manifestation of vitamin E-deficiency in the chick is the first-week embryonic mortality. The deficiency can be greatly reduced by supplying vitamin E in the poultry diet.

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LEUKOPENIA AND ANEMIA IN THE MONKEY RESULTING FROM VITAMIN DEFICIENCY[1]

PAUL L. DAY, WILLIAM C. LANGSTON AND CARROLL F. SHUKERS
Departments of Physiological Chemistry and Anatomy, School of Medicine, University of Arkansas, Little Rock

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The discovery that cataract may be produced in rodents by restricting them to a diet deficient in vitamin G (Day, Langston and O'Brien, '31; O'Brien, '32; Langston and Day, '33, '34; Langston, Day and Cosgrove, '33; Yudkin, '33; Day and Langston, '34) led us to attempt similar experiments with monkeys. Before advanced cataractous changes developed, however, the monkeys died of a fulminating blood disease, characterized by marked anemia, leukopenia, gingivitis, and diarrhea. It is the purpose of this paper to report briefly the production of this hitherto unrecognized deficiency syndrome.

EXPERIMENTAL

The young rhesus monkeys (Macaca mulatta) used in these experiments were purchased from animal dealers. At the time they were placed on the experimental diet they weighed between 2.0 and 2.7 kg. each, and although histories were not available, from their weights and condition of dentition (Hartman and Straus, '33) they appeared to be about 2 years old. After a period of conditioning and observation, they were given a diet similar to that used in the production of cataract in rats. The daily ration for each monkey was as follows:

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[1] The expenses of this investigation were defrayed in part by a grant from the Committee on Scientific Research of the American Medical Association.

	Grams
Casein, Sherman and Spohn ('23)	10
Whole wheat, ground	15
Polished rice	30
Cod liver oil[3]	3
Salt mixture, Osborne and Mendel ('19)	4
Sodium chloride	2
Orange	4

The rice, casein, salt mixture and salt were cooked with sufficient water in a double boiler for 1½ hours, the wheat was then added and the cooking continued for another ½ hour. The cod liver oil was mixed with the ration just before feeding it. The orange was fed separately.

This diet is believed to be adequate, qualitatively and quantitatively, with respect to protein, inorganic elements, fatty acid, vitamins A, B(B_1), C, and D, but is deficient in vitamin G(B_2) and possibly some of the less well-known vitamins. Since Harden and Zilva ('20) found that 2 cc. of orange juice daily protected monkeys from scurvy, our animals received an adequate amount of ascorbic acid, yet the 4 gm. of orange given them supplied less than one Bourquin-Sherman unit of vitamin G (Day and Darby, '33). We are not convinced that the blood picture in the monkey here reported was the result of deprivation of the rat-growth vitamin G. It does appear probable, however, that the symptoms which developed were the result of a lack of some vitamin or vitamins.

The animals were observed daily, and at intervals of 1 week, or oftener, were taken from their cages and bled from the ear for hematological and chemical analyses. Total leukocyte and erythrocyte counts were made in duplicate, and smears for differential counts were made in the usual manner and stained with Wright's stain. Hemoglobin was determined by the acid hematin method of Newcomer, using a standard glass color disc for the colorimetric comparisons. Blood glucose was determined by the micro method of Folin ('28), and cholesterol by the Leiboff method ('30). Urea was estimated by the micro method of Keller (Kolmer and Boerner, '31). The animals were given careful physical examinations, including observations of the mouth. Ophthalmoscopic examinations were frequently made. After the onset of the hypocythemia, blood determinations were made at semiweekly or daily intervals. At death autopsies were performed, and tissues were taken for histological examination. The pathological findings, including eye and bone marrow changes, will be reported later.

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[3] Supplied by the E. L. Patch Company, Boston.

RESULTS AND DISCUSSION

In general, the monkeys ate the diet well, and although all showed a loss of appetite during the final stages of the disease, we feel that the blood changes were not due to anorexia, since the anemia and leukopenia preceded the loss of appetite. The animals on the deficient diet all lost weight, and showed marked emaciation before death. With one exception, the monkeys all developed a diarrhea within a short time after being placed on the diet. In some cases the diarrhea was intermittent, and in others continuous, with yellow or greenish, watery stools. We experienced some trouble with dysentery in our animals—a condition not uncommonly encountered by investigators working with imported rhesus monkeys. Since the deficiency appeared to predispose to an attack of a latent dysentery, stool cultures were made at frequent intervals, and animals showing positive tests for pathogenic organisms were excluded from the series.[3] Such animals usually died of an acute attack of dysentery, with large amounts of blood and mucus in the stools. The diarrhea caused by the deficiency, uncomplicated with dysenteric infections, was not characterized by blood and mucus, however, and did not appear to produce any marked ill effects upon the monkeys.

[3] A number of animals that died with clinical symptoms of dysentery yielded positive stool cultures for Shigella paradysenteriae, Hiss variety. One yielded a positive culture for Sonne variety.

In a series of seven monkeys dying of the deficiency, uncomplicated by any known dysentery or other infections, hematological data were obtained upon six of the animals, and blood chemical analyses were made upon five. Although there was some variation between individuals, the disease ran a rather typical course in all. As indicated in the introduction, the outstanding features were anemia and leukopenia, accompanied by, or followed by, an ulcerative gingivitis. The hematological and blood chemical findings and oral lesions are discussed below under the appropriate headings.

Hemoglobin. Normal values for hemoglobin in the monkeys used in these experiments ranged from 10.0 to 14.0 gm. per 100 cc. of blood, although most normal values came within the somewhat narrower limits of 11.0 and 13.0 gm. Monkeys on the deficient diet, after a variable period in which the hemoglobin remained normal, showed a progressive and usually rapid decrease in pigment. The extent of diminution of hemoglobin depended somewhat upon the length of survival of the animal after the anemia developed. The average final content of hemoglobin was 7.10 gm., but one went as low as 2.27 gm. per 100 cc. of blood.

Erythrocyte count. Normal erythrocyte counts on the young monkeys were found to be between 4.5 and 6.0 million per cubic millimeter. In the experimental animals there occurred a decrease in red corpuscles coincident with and paralleling the decrease in hemoglobin. The count before death was less than 3.0 million cells in every case, and in one animal dropped to less than one million (955,000) erythrocytes per cubic millimeter of blood.

Leukocyte count. The normal range of white cells in young monkeys is rather high and quite variable in the experience of Wills and Bilimoria ('32), and this was found to be the case in our animals also. The monkeys on a mixed stock diet, and normal controls receiving the deficient diet plus yeast, had total white counts between 10,000 and 30,000. There was a marked difference between animals, and also a distinct variation from week to week in any one individual. Because of this wide variation and the lack of a definite normal standard, it is difficult to say just when a leukopenia developed in any given case. However, a definite drift downward usually started at about the same time as the decrease in hemoglobin and erythrocytes began. Minimum counts were below 5500 in every case; four monkeys out of six had white counts below 2000, and one animal had a final total leukocyte count of only 695 cells per cubic millimeter.

The differential counts were so variable that it is difficult to make any broad statement that holds true for all the animals. In general, however, as the leukopenia developed, the decrease in white cells appeared to be more at the expense of the neutrophils than of the lymphocytes. That is, there was frequently a relative neutropenia together with a relative lymphocytosis, although there was, of course, an actual lymphopenia as well as an actual neutropenia. One animal in its final count exhibited 94 per cent lymphocytes and 4 per cent neutrophils. The differential counts will be given in detail in a later report.

Blood cholesterol. Monkeys on a normal stock diet (grains, sunflower seeds, bread, fruits and vegetables) had cholesterol values as high as 200 mg. per 100 cc. of blood, but a normal control receiving the deficient diet supplemented with yeast showed cholesterol as low as 130 mg. Cholesterol in the blood of animals on the deficient diet fell progressively, decreasing to 100 mg. or less in all five of the animals on which determinations were made, and in one animal going as low as 62 mg. per 100 cc. of blood.

Blood glucose. The animals were found to have blood sugar levels between 100 and 140 mg. per 100 cc., although excitement or exercise resulted in glucose levels higher than 140 mg. The experimental animals had normal blood sugar values until the last day or two of life, when there was a decrease below normal, going as low as 60 mg. before death. The blood samples were always drawn before the daily feeding, and carbohydrate tolerance tests were not made.

Blood urea. The normal values were found to be between 12 and 24 mg. urea N per 100 cc. blood. There was no significant variation from normal in the experimental animals until the last 2 days of life, at which time there was an increase. Blood samples taken from the moribund animals were found to contain between 40 and 47 mg. urea N per 100 cc. This terminal increase possibly is not important, as it may have been due to failing circulation. Sufficient blood was obtained from the heart of two animals at death to make determinations of creatinine possible. Each had less than 2.0 mg. creatinine per 100 cc. of blood, so it may be concluded that there was no retention of this substance.

Oral lesions. The only consistent physical finding was an ulceration of the margins of the gums. This lesion usually developed within a short time after the onset of the leukopenia, first appearing as a slight recession of the gums, and later as a distinct yellowish ulceration. Upon wiping off the necrotic material with a cotton swab, mild bleeding resulted. This ulceration usually developed along the incisors first, and in several animals caused the teeth to fall out. In one monkey the ulcerated region was chiefly along the canine and molars, and progressed until a large area of bone was exposed. The gums were pale and there were no marked spontaneous hemorrhages. No other oral lesions were observed.

With the exception of a single, small, perforating ulcer on the paw of one animal, no skin changes were seen.

The survival period on the deficient diet varied from 26 to 81 days, with an average of 56 days. Since a normal control receiving the deficient diet plus dried brewers' yeast has lived more than 200 days without showing any of the signs discussed above, it appears that this progressive hypocythemia was due to a deficiency of some substance which is found in yeast. It seems probable that the syndrome was due to a lack of some component of the vitamin B complex.

Since black tongue in dogs may be produced by feeding a diet similar to the diet we used, it would have been natural to expect an analogous condition in the monkey. However, because of the nature of the oral lesions and the absence of dermatitis, it is apparent that the condition in the monkey herein reported does not clearly resemble either black tongue or pellagra.

SUMMARY

Young monkeys (Macaca mulatta) were given a diet believed to be adequate with respect to protein, inorganic elements, fatty acid, vitamins A, $B(B_1)$, C, and D, but deficient in vitamin $G(B_2)$ and possibly deficient in other less well-known organic substances which may be essential to the nutrition of the monkey. After a varying period on the diet, the animals developed a fulminating, fatal blood disease, characterized by anemia and leukopenia. Ulceration of the gums was a consistent accompaniment of the hypocythemia, and diarrhea was common. There was a progressive decrease in blood cholesterol, but no significant change in the blood sugar level. Blood urea remained normal until the last 2 days of life, at which time the level of this substance became somewhat elevated. There was no retention of creatinine.

The deficient diet supplemented with dried brewers' yeast supported good growth and prevented the deficiency syndrome.

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THE USE OF POLARIZED LIGHT IN THE STUDY OF MYELIN DEGENERATION

II. THE DEGENERATION OF MYELINATED NERVES IN AVITAMINOSIS A IN THE WHITE RAT

H. E. SETTERFIELD AND T. S. SUTTON
Departments of Anatomy and Animal Husbandry, The Ohio State University Columbus

FOUR FIGURES

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INTRODUCTION

Early in 1927 symptoms indicative of nervous disorders were first noted in certain groups of rats in our colony which were being used in vitamin A assays. The highest incidence of the symptoms in question was in groups receiving the lower levels of vitamin A supplement after being completely depleted of vitamin A. Animals which were completely depleted of vitamin A were characterized by extreme xerophthalmia, emaciation, great weakness and early death. After from 45 to 50 days on a vitamin A free diet, plus low levels of vitamin A supplement after depletion, these rats showed symptoms not previously described as associated with avitaminosis A.

The early symptoms in these animals are characterized by a peculiar weaving, unsteady gait in the hind limbs. There is a definite lack of coordination rather than an actual paralysis in early stages. Frequently in moving about the limb or limbs are abducted to a degree greater than normal. There soon develops a relaxed condition of the leg: the limb being partly extended at all times while walking. The animal seems unable completely to flex the knee and hip joint. If the rat is held up in a vertical position the affected leg is allowed to

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hang in a relaxed, extended posture while the unaffected one is drawn up close to the trunk. The condition is usually unilateral and in cases of bilateral involvement the symptoms are always more pronounced in one leg. There is, however, no predilection for either side. We have seldom found the condition affecting the front legs. We believe this is due to the fact that the rats succumb before the progress of the disease has reached them rather than any limitation of the disease itself to the hind legs.

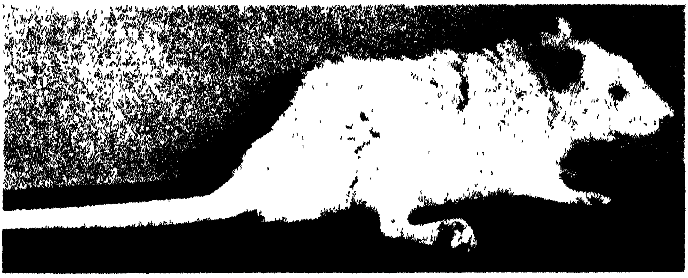

Fig. 1 Rat 724. Diagnosis ++++. Weight 104 gm. Weight of positive control in same group 214 gm.

This rat was fed a vitamin A free diet for 35 days. At that time it had stopped gaining weight and had xerophthalmia. It was then given 30 mg. butter fat per day. At the end of 10 days it showed symptoms of nervous disorder. These progressed steadily until the animal was killed after 4 weeks on the supplemented diet (30 mg. of butter fat is less than a unit dose). (Table 1.)

Note: roughened fur, swollen conjunctiva, abduction of leg, flexion of toes, relaxed position of posterior half of the body. Not apparent in the picture: flaccid and atrophic muscles of the thigh and leg.

With the progress of the disease the ability to use the affected parts diminishes. The rat walks on its heels with little or no use of the digits. The muscles of the thigh and leg become flaccid and then atrophied. The digits are carried always in the flexed position. We have never noted a convulsive or spastic condition. In moving about the leg is frequently abducted to such a degree that the animal walks on the side of the heel rather than on the sole of the foot. The body is carried low, the belly almost or quite touching the floor. The legs are spread apart and extended. The muscles of the tail are often affected and sometimes atrophy. In a few cases the tail muscles on one side only are affected and the tail is then curved away from the affected side. A painful stimulation of the tail or toes brings only a feeble response or none at all. The animals frequently chew their own tails or feet. In a few cases individuals have chewed off the whole tail or a whole foot up to the ankle joint. The final result of the disease is an irreparable atrophy of the muscles of the thigh and leg. Animals which have suffered such atrophy do not recover control or replace muscle fibers even though a complete diet brings about complete recovery from all other symptoms of avitaminosis A.

Fig. 2 Section of a normal sciatic nerve of white rat. This animal had been on a complete diet. Photographed in polarized light, between crossed nicols, at a magnification of 1200.

Note: The dark lines are axis cylinders, the light areas are myelin sheaths. Normal node of Ranvier in lower right.

We suspected that the spinal nerves to the parts affected were involved in the disease and were degenerating due to some unknown effect of the vitamin deficiency. It was in an attempt to describe the effects of avitaminosis A on the peripheral nerves of the rats that this study was begun.

In the early stages of the work the sciatic and femoral nerves and spinal cords were removed and some of them fixed in potassium bichromate and further prepared by the Marchi method. Others were fixed in 10 per cent formalin and later frozen sections of them were stained with Sudan III or Scharlach R. We found, as had Duncan ('31) and Prickett ('34), that in the Marchi preparations blackened droplets

Fig. 3 Diagnosis ++++. This rat (701) received the same diet as 724. It was in about the same physical condition except that the xerophthalmia had improved somewhat more than in the other rat. Not quite so many fibers are involved but those affected are in a later stage of degeneration. Compare the greatly swollen fibers with those in figure 7 of preceding paper.

Photographed in polarized light between crossed nicols at a magnification of 1200.

were visible in normal controls as well as in our diseased animals. Likewise the fat stains, Scharlach R and Sudan III were unsatisfactory since they depend on relative intensity of color and since this varies with the temperature, thickness of section, concentration of stain, degree of degeneration, and length of time stained. While we were satisfied that the nerves were affected we could not demonstrate it with certainty in early stages by any of the three methods named.

Fig. 4 Diagnosis ++++. Rat 755. This rat was depleted in 35 days and then received 30 mg. of butter fat per day for 3 weeks. It showed nervous symptoms for 1 week before it was killed. The appearance of the fibers resembles those in plate 3 but more fibers in the early stages of degeneration are present. One in the center of the field is in a late fragmenting stage.

Photographed in polarized light between crossed nicols at a magnification of 1200.

In searching the literature for a more delicate technic the statement in Lee ('21) by Cramer and Lee concerning the reactions of various lipids was noted. Cramer there states that the true fats, or triglycerides, are isotropic while the phospholipids are doubly refractive, or anisotropic. It is therefore possible to distinguish these two groups of substances by the use of the polarizing microscope.

Following this lead and the other facts noted in the first paper of this series we applied this test for the presence of the degeneration products of myelin (triglycerides) in the nerves from our rats. Finding that we could observe very slight changes from the normal we proceeded to establish the technic for the study of degeneration by using it in a series of animals in which experimentally produced Wallerian degeneration was present. This work constituted the subject matter of the first paper of this series (Setterfield and Sutton, '35).

MATERIALS AND METHODS

Feeling that our experience with the new technic was too limited to include the central nervous system in our observations, we restricted our material to the spinal nerves related to the affected parts. These were prepared and studied as described in the preceding paper. We studied nerves from a total of eighty-five rats on various levels of vitamin A supplement as well as normal controls.

It was found that among forty-eight rats on an extremely low level of vitamin A supplement after depletion (30 mg. butter fat per day) 97.9 per cent of them developed nervous symptoms and soon died. Of these we observed nerves from twenty-one. Nine of them were diagnosed as +++[1] while twelve were ++++. It was subsequently found that this was also true when the rat received 1 mg. cod liver oil or 0.4 gamma of carotene per day after depletion (table 3). Among twenty rats on an adequate[2] vitamin A supplement (60 mg. of butter fat per day) after depletion, none of the animals developed symptoms (table 1). However, later studies of the time of appearance of degeneration compared with the time symptoms developed showed that degeneration had begun but had not proceeded far enough to produce external signs. Duncan ('30) has stated that degenerating fibers are always found in the peripheral nerves of the normal rat and that in rats low in vitamin A the rate of degeneration is increased. However, the rat in this study that showed the greatest number of degenerating fibers was vitamin A deficient and free from infection, indicating that the degeneration was not due to infection. He has estimated that less than 1 per cent of nerve fibers in the sciatic nerve of a normal rat may be degenerating at any one time. The rate of degeneration increases with age. However this percentage represents less than one fiber in a whole sciatic nerve. Degeneration in our rats at the time under discussion (⊥ or +) has gone much further, there being from one to four fibers

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[1] The signs ⊥, +, ++, +++, and ++++ are used to describe the severity of degeneration and roughly the extent of involvement of the nerve fibers in a nerve. ⊥ refers to a condition where there is only a slight swelling of a few fibers and no isotropic material. + refers to the stage when one or two fibers in the section are greatly and irregularly swollen and are beginning to lose their birefringence. ++ is a stage where approximately every third fiber is degenerating, greatly swollen and showing extensive isotropic masses

In the +++ stage about two out of three fibers are badly degenerating. In the ++++ stage every fiber is affected more or less, ranging from early degeneration in some to extreme fragmentation and isotropic condition in others.

All these stages are arbitrary and not exact. Their value is relative and they are simply useful terms in describing the course of degeneration.

TABLE 1

The occurrence of nervous symptoms in rats receiving a vitamin A deficient diet, supplemented with varying levels of butterfat after cessation of growth and appearance of xerophthalmia

NUMBER OF RATS	LEVEL OF BUTTERFAT SUPPLEMENT	PER CENT OF RATS SHOWING NERVOUS SYMPTOMS	MICROSCOPICAL DIAGNOSIS
20	60 mg. daily	00	No microscopic diagnosis
48	30 mg. daily	97.9	The femoral and sciatic nerves from 21 of these rats were examined microscopically 9 showed +++ degeneration 12 showed ++++ degeneration

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[2] By an adequate supplement of vitamin A is meant an amount per day sufficient to produce as great an average increase in weight per day as is shown by normal stock rats of the same age which have not been depleted and are on a complete diet.

in an average high power field definitely degenerating at this time. We have not made total counts to show the exact extent to which degeneration may go before external signs of the loss are visible.

TABLE 2

The progress of nerve degeneration in avitaminosis A. (Basal diet —A, 0.5 gm. yeast 2.6 units of vitamin D (viosterol) daily)

RAT NO.	DAYS ON DEFICIENT DIET	DURATION OF XEROPHTHALMIA	CHANGE IN WEIGHT FROM MAXIMUM	MICROSCOPIC DIAGNOSIS	
				Femoral	Sciatic
C802	28	No xerophthalmia	Still gaining	Normal	⊥
C805	28	No xerophthalmia	Still gaining	Normal	⊥
C804	31	No xerophthalmia	Still gaining	Normal to ⊥	Normal to ⊥
C807	31	No xerophthalmia	Still gaining	Normal	Normal to ⊥
C803	34	Incipient xerophthalmia	Still gaining	+	+
C806	34	Incipient xerophthalmia	Still gaining	+	+
C812	34	Incipient xerophthalmia	Still gaining	+	+
C813	34	Incipient xerophthalmia	Still gaining	+	+
C668	39	3 days	Still gaining	+	+
C673	39	3 days	Still gaining	+	+
C667	43	7 days	— 5 gm.	+	++
C675	43	7 days	— 9 gm.	++	++
C671	50	7 days	— 12 gm.	+++	+
C670	56	10 days	— 34 gm.	+++	+++
				(wobbly gait in rear limbs)	
C672	56	10 days	— 16 gm.	+++	+++
				(impaired use of right foot)	
C674	60	20 days	— 25 gm.	++++	++++
				(impaired use of left foot)	
C666	72	26 days	— 21 gm.	+++	++++
				(wobbly gait, poor coordination)	

The study referred to above, summarized in table 2, was the diagnosing of nerves from rats on a deficient diet beginning 8 days before the appearance of xerophthalmia or cessation of growth. In these the diagnoses were ⊥ and + of degeneration even though there were no external signs whatever of vitamin deficiency. By the time the rats began to lose weight the amount of degeneration had increased to ++

TABLE 3

Progress of degeneration is arrested by adequate dosages of vitamin A supplements administered after xerophthalmia develops. (Unless otherwise designated, all rats were killed after 8 weeks of supplement feeding.) (Basal diet —A_{11} 0.5 irradiated yeast)

RAT NO.	SUPPLEMENT	CHANGE IN WEIGHT DURING SUPPLEMENT FEEDING	DURATION OF NERVOUS SYMPTOMS BEFORE KILLING OR DEATH	MICROSCOPIC DIAGNOSIS (FEMORAL AND SCIATIC NERVES)
C688	30 mg. B.F.	— 5[1] (42 days)	7 days	+++
C676	30 mg. B.F.	— 20[1] (29 days)	8 days	++++
C694	30 mg. B.F.	— 16[1] (43 days)	21 days	+++
C682	30 mg. B.F.	— 2	21 days	+++
C690	1.0 mg. C.L.O.	— 6[1] (46 days)	14 days	+++
C677	1.0 mg. C.L.O.	+ 16	21 days	+++
C695	1.0 mg. C.L.O.	— 10[1] (41 days)	13 days	+++
C686	1.0 mg. C.L.O.	+ 28	28 days	++
C692	0.4r carotene	+ 34	14 days	++
C678	3 weeks 0.8r	+ 51	28 days	+++
C696	Carotene 5 weeks	+ 25	42 days	+++
C684	Carotene 5 weeks	+ 54	Kept for demonstration	
C679	90 mg. B.F.	+ 56	No symptoms	+
C685	90 mg. B.F.	+ 56	No symptoms	⊥
C680	3.0 mg. C.L.O.	+ 50	No symptoms	+
C683	3.0 mg. C.L.O.	+ 66	No symptoms	⊥
C681	1.2r carotene	+ 52	No symptoms	++
C687	1.2r carotene	+ 75	No symptoms	⊥
C699	1.2r carotene	+ 64	Kept for demonstration	

[1] Rat died before the end of the experiment.

and from that time progressed to +++ during the first 7 days of xerophthalmia and to ++++ by 20 days of xerophthalmia. By the time degeneration had reached +++ in the sciatic the animal's locomotion was greatly impaired and both sensibility and motor control greatly diminished (table 2).

Progress of degeneration can be arrested by adding adequate amounts of vitamin A to the diet of the depleted animals. In rats receiving only 30 mg. of butter fat per day the degeneration progressed steadily to ++++ and the early death of the animals. However if, when xerophthalmia appears (i.e., at least 6 days after degeneration begins) the diet is supplemented by 90 mg. of butter fat per day the degeneration remains at the ⊥ or + stage which is always present when xerophthalmia appears. These facts are summarized in table 3.

The observations cited above demonstrate the presence of degeneration in amounts and at times not observable by any other technic.

All the diagnoses in the eighty-five rats studied were made objectively, the observer in no case being aware of the physical condition of the animal from which the nerves were taken. More detailed discussion of the nutritional aspects of these vitamin A studies has been published under appropriate title (Sutton, Setterfield and Krauss, '34). Our purpose in this paper is to describe the application of the polarized light technique to the study of very early and very small amounts of degeneration in spinal nerves.

CONCLUSIONS

1. Polarized light has been used to study qualitatively, quantitatively and chronologically the progress of myelin degeneration in rats on diets deficient in vitamin A and diets supplemented by various amounts of vitamin A.
2. Degeneration in the sciatic and femoral nerves begins from 3 to 6 days (at least) before there is any external sign of avitaminosis A.
3. Between one-fourth and one-third of the fibers are in the process of degeneration before there is any external sign of loss of sensibility or motor control in rats suffering from avitaminosis A.
4. Adequate dosages of vitamin A administered after depletion arrested the progress of degeneration.

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THE EFFECT OF PROTEIN AND AMINO ACID METABOLISM ON THE UREA AND XYLOSE CLEARANCE

ROBERT F. PITTS
Department of Physiology, New York University and Bellevue Hospital Medical School, New York City

THREE CHARTS

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Investigators who have studied the kidney under suitably controlled conditions have frequently remarked on the variations in renal function that seem to be of a physiological nature. Most noteworthy in this connection is the influence of the maintenance diet, as well as the immediate influence of the ingestion of food. Addis and Drury ('23) observed that the rate of excretion of urea relative to the blood urea is increased in man after feeding milk or glutamic acid. Jolliffe, Shannon and Smith ('32) have observed that urea, xylose and creatinine clearances in the dog are not only elevated by the feeding of meat, but are greater when observed in the post-absorptive condition in dogs which are maintained on a meat diet than in the same dogs maintained on a low protein diet. These investigators have concluded that the functional changes are related to changes in the rate of glomerular filtration.

The experiments reported in the present paper are intended to examine to what extent these variations in activity are related to meat, as compared with other proteins; to what extent they are dependent on digestion and absorption on the one hand or metabolism on the other; and to what extent they can be correlated with any specific phase of protein metabolism.

METHODS

Female dogs were used throughout. Urine was obtained by catheter with care to insure complete emptying of the bladder, and blood was drawn from the external jugular vein. Urea was determined by the gasometric method of Van Slyke ('27), and xylose as described by Jolliffe, Shannon and Smith ('32).

The basal diet consisted of 100 gm. of cracker meal, 30 gm. of cane sugar, 30 gm. of butter, 10 cc. of cod liver oil, 5 gm. of dry irradiated yeast and 0.6 gm. of Cowgill's salt mixture, moistened with warm water and well mixed. The dogs were fed at about 5 P.M. and, unless otherwise stated, the experiments were performed 16 to 18 hours after feeding.

RESULTS

It is evident that results obtained on different animals can be compared only with great uncertainty, and for this reason we have made our observations as far as possible on the same animals under a variety of conditions. It has been impossible to present in tabular form the extensive data of our experiments, and for this reason representative experiments have been summarized graphically in the appended charts. These charts are uniform in having the xylose clearance (upper line) and the urea clearance (lower line) plotted against time expressed in units of 100 minutes. The data at the bottom of the charts state the types of diet and the time which the dogs had been maintained thereon. The figures above these data state the average ratio: urea clearance/xylose clearance. Every experiment, unless otherwise noted, was begun with a series of three control observations made in the post-absorptive condition at least 16 hours after the last meal. The dog was then fed or injected, as the case might be, and the clearances were observed for some time thereafter. The dotted lines indicate periods in which no urine collections were made, and the figures written beside arrows pointing to these lines indicate instances where there was a lapse of time that could not be expressed on the charts. The urine collection periods averaged 30 minutes each and the urine flows ranged from 1 to 6 cc. per minute, with the exception of experiment 78 in which they ranged from 0.59 to 0.93 cc. All of these are above the augmentation limit.

Experiment 32 (chart 1) is a series of control observations on dog 7 made in the post-absorptive state after 14 days on a basal diet. The average data (urea clearance/xylose clearance = X) for five consecutive periods are 13.9/19.7 = 0.70. Five days later (experiment 33), this dog was again observed in the post-absorptive state: 16.5/20.5 = 0.80. The dog was

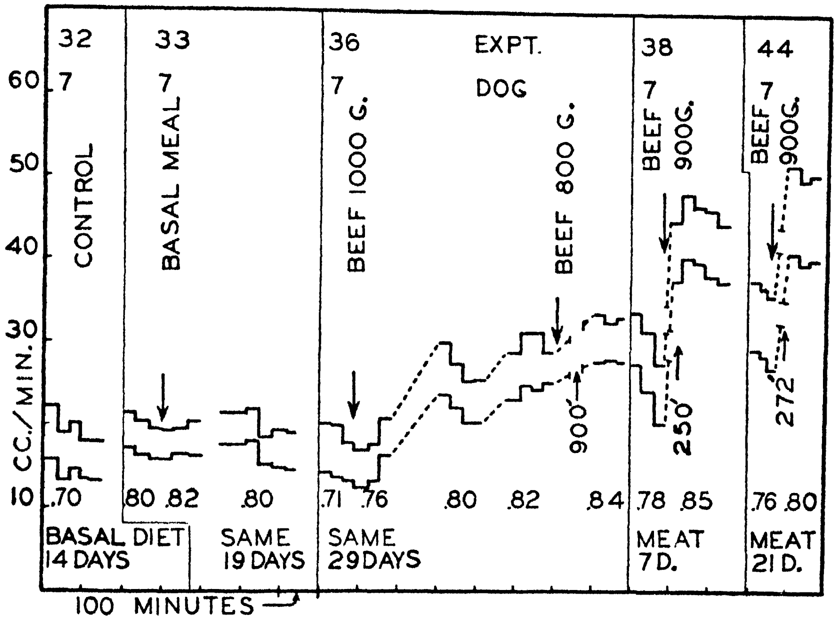

Chart 1

then fed a basal meal without any significant change in the average clearance levels of either urea or xylose. Ten days later (experiment 36), this experiment was repeated, substituting 1000 gm. of raw chopped beef for the basal meal, whereupon the clearances rose from a control level: 13.9/19.6 = 0.71, to: 24.7/30.3 = 0.82. Another meal of 800 gm. of raw beef raised the clearances to: 28.1/33.4 = 0.84. After a week on a meat diet consisting of 900 gm. of raw chopped beef daily, both clearances (experiment 38) remained at an elevated level in the post-absorptive condition (24.3/31.2 = 0.78), and now a further feeding of meat increased them again: 39.1/46.3 = 0.85. This is, respectively, 2.81 and 2.36 times the post-absorptive basal-diet level obtaining a week earlier, before the first meat meal. After 2 more weeks on a meat diet there was a still further increase in the post-absorptive clearances: 28.3/37.2 = 0.76; and after feeding meat these clearances again rose in the usual manner to: 40.6/51.1 = 0.80, or to 2.92 and 2.60 times the original basal level. These results confirm the observations of Shannon, Jolliffe and Smith ('32) that the urea and xylose clearances tend to be low and constant in the post-absorptive state while the animal is on a basal diet; that the ingestion of a protein-poor meal does not significantly elevate these clearances; that the feeding of meat produces a rise in both clearances within a few hours; and that a higher post-absorptive level is maintained on a meat diet than on a protein-poor diet. The change in the urea and xylose clearances is relatively not so great in our experiments as was observed by the above investigators, however, but much greater than those that have been observed by Cope ('33) and by Goldring, Razinsky, Greenblatt and Cohen ('34) in man on low and high protein diets.

Experiments 40, 41, 42 and 45 (chart 2) show the effects of feeding pure casein for 7 days. Two hundred and thirty grams of casein, suspended in warm water, were administered by stomach tube each day. The urea and xylose clearances in the post-absorptive state rose to 74 and 60 per cent above the basal level and increased to 109 and 89 per cent above the basal level after a casein meal. These changes are somewhat less than were obtained with meat, but leave no doubt that casein acts qualitatively in the same manner as meat.

On the day previous to experiment 41 and after the control observations of experiment 40 had been made, the dog refused to eat and there was a significant drop in the level of the urea and xylose clearances, but these were returned toward the normal post-absorptive level by feeding the basal meal (see chart). It appears that the post-absorptive level obtained on a protein-poor maintenance diet is a basal level only in the sense that it is relatively constant and not in the sense that it is minimum.

To exclude the participation of the gastro-intestinal tract in these phenomena, metabolism of endogenous protein was stimulated by thyroxin. Experiment 46 (chart 2) shows that thyroxin in doses of 10 mg. has no appreciable effect on renal activity the first day, but experiment 47 shows that a sharp rise occurs on the second day; after three additional 10 mg. doses of this drug on alternate days, total increases of 45 and 53 per cent, respectively, above the basal clearances of urea and xylose were reached.

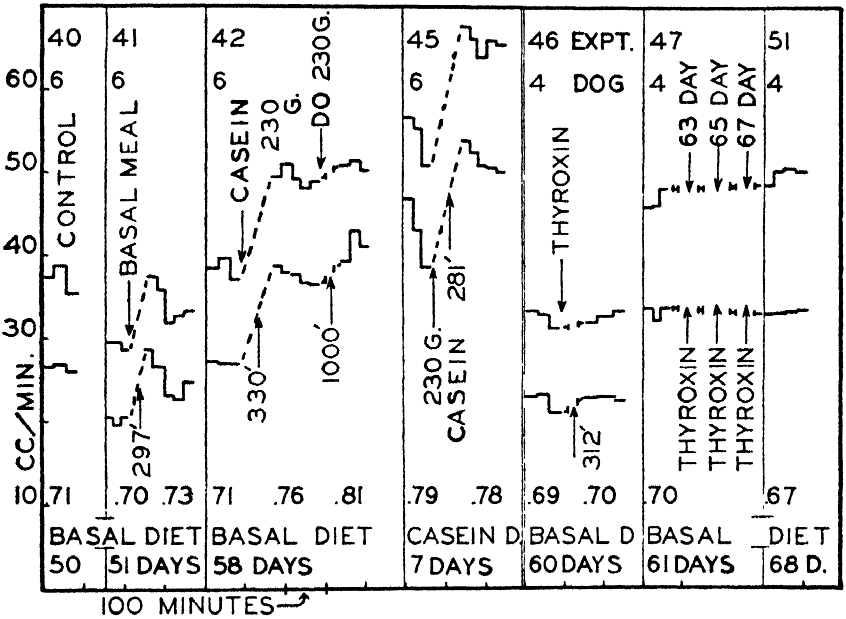

Chart 2

It is well known that phlorizin increases the protein metabolism of a fasting dog and we have examined the effects of the drug in the above respect. These experiments are not included in the appended charts. In experiment 61 four control periods yielded the following values on dog 12, which had been on a basal diet for 5 days: (urea clearance/xylose clearance = X) 29.3/41.3 = 0.71. After 3 days of fasting, four periods yielded average figures of 27.2/36.6 = 0.74. Two hundred milligrams per kilogram of phlorizin in sodium bicarbonate solution were administered subcutaneously at noon of the day after this experiment and again at midnight. The next morning four periods yielded the average figures 46.5/62.9 = 0.74. Similar experiments with dog 13 on basal diet for 5 days yielded for four control periods 33.3/42.9 = 0.78. The dog was fasted the next day and given phlorizin as above. The following day four periods yielded 35.7/50.1 = 0.71. Another 200 mg. per kilogram of the drug were given subcutaneously in the afternoon and an additional 200 mg. were given at 2 A.M. the next day. Eight hours later, four periods averaged 44.3/62.2 = 0.72. It would appear from these experiments that an increased metabolism of body protein, as well as an increased metabolism of exogenous protein, acts in the direction of increasing renal function. This fact may be significant in the observations made by Goldring ('31) on the urea clearance of man during the acute febrile stage of rheumatic infection.

In order to test whether this increased renal activity is a specific effect of amino acids, a series of experiments were performed in which glycine was incorporated in the diet. Experiments 78 and 79 (chart 3) show that glycine increased the urea and xylose clearances immediately when administered by mouth, and also in the post-absorptive condition when incorporated in the diet to the extent of 25 gm. per day The effects of subcutaneous and intravenous administration of amino acid were examined in experiments 85 and 86. In these experiments both clearances essentially reached their peak values a few minutes after administration of glycine; the blood urea, however, showed no significant elevation at this time and was still rising at the conclusion of the observations, indicating that the final metabolism of the amino acid is not the effective factor in promoting renal activity. The fact that the clearances rose simultaneously with the blood amino acid, as shown by analyses of the latter that are not included here, indicates that the amino acid may act directly. There is, however, no proportionality between blood amino acid-nitrogen and increased renal activity.

Experiments 89 and 91 show that glycylglycine and alanine have the same action upon the urea and xylose clearances as glycine, and make it impossible to suppose that the stimulating effect is specific. Glucose, on the other hand (experiment 94), has no effect whatever.

Additional experiments, not to be reported here, have shown that glycolic and pyruvic acids increase both clearances from

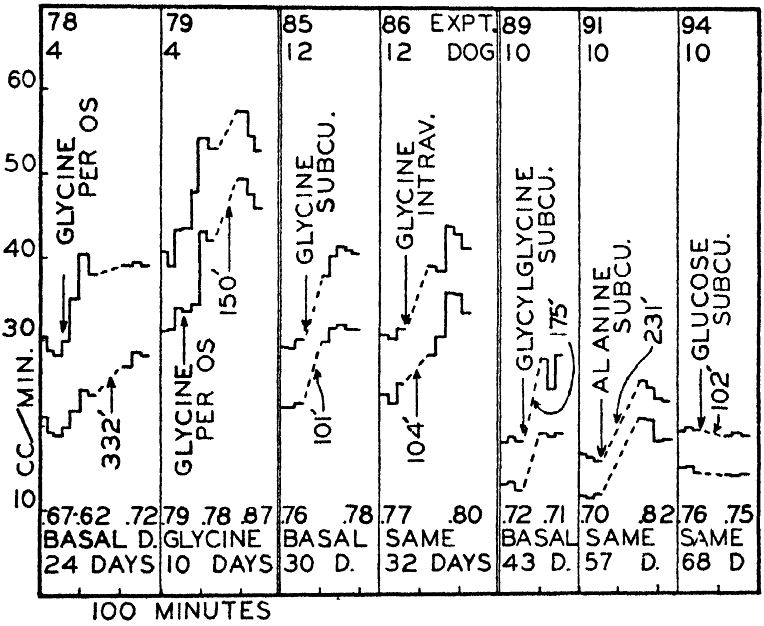

Chart 3

10 to 50 per cent. It seems possible, therefore, that hydroxy acids produced during the metabolism of amino acids may be responsible in part for the increase in renal activity, and consequently it is unwise to attribute prepotency to the amino acid molecule itself.

Other experiments performed in this laboratory have shown that the administration of a salt mixture similar in amount and in composition to the salt content of the meat diet is without effect on the urea and xylose clearances. Large amounts of sodium sulfate administered intravenously have likewise been shown to exert no appreciable effect on these clearances. It should be reiterated that in all the experiments referred to above, the control observations, as well as the experimental ones, were made with the urine flow above the augmentation limit.

DISCUSSION

If the xylose[1] clearance be accepted as a measure of glomerular filtration, as recommended by Jolliffe, Shannon and Smith ('32), it is evident that the increased renal function can in large part be accounted for by an increase in the latter. It is not possible to explain the facts that the feeding of meat, casein and glycine increase the urea clearances relatively more than the xylose clearance, whereas the administration of thyroxin and phlorizin has no such effect. The explanation of these facts cannot be obtained until an explanation is available of how the glomerular clearance itself is increased, or until it is known why the urea clearance is invariably lower than the xylose clearance. Richards and Plant ('22), Richards and Schmidt ('24), Bieter ('29), White ('30), Khanolkar ('22), Hayman and Starr ('25) and Sheehan ('31) have advanced evidence that the activity of amphibian and mammalian glomeruli is variable, but the reasons for, and mechanism of, this variability are not yet clear.

Brühl ('28) and Watzadse ('28) describe experiments in which failing glomerular circulation in an isolated perfused frog kidney is revived by the addition of glycine. These authors find that amino acids in general, dipeptides, amino acid amides, ereptone, and peptone all increase glomerular activity, and they maintain that the presence of some amino-nitrogen containing substance is necessary for continued glomerular circulation. These observations are of particular interest in view of the results which have been reported here.

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[1] Evidence obtained in this laboratory since this work was completed suggests that there may be a small fraction of the filtered xylose that is actively reabsorbed (compare Shannon, '34). No final opinion on this matter, so far as the dog is concerned, can be given at the present time, but if such proves to be the case the fact of this reabsorption would not modify the significance of the changes in clearance described here.

SUMMARY

The post-absorptive urea and xylose clearances in dogs maintained on a basal diet are low and constant from day to day, and feeding the basal diet has itself little effect on these clearances.

The feeding of meat produces a gradual and prolonged post-prandial rise in both clearances relative to the levels existing before the meal. When meat is used as a maintenance diet over a period of 2 or 3 weeks the post-absorptive clearances are elevated, while the post-prandial effects of eating meat are not obliterated.

A casein diet has qualitatively the same effect in elevating the urea and xylose clearances as has meat. The administration of thyroxin to a dog maintained on a basal diet, and the administration of phlorizin to a fasting dog, likewise elevate the urea and xylose clearances above their respective post-absorptive levels. These facts indicate that protein metabolism as such, whether endogenous or exogenous, is responsible for the increase in renal function.

Feeding a basal diet in which a part of the carbohydrate has been replaced by glycine has the same qualitative effect upon the post-absorptive and post-prandial urea and xylose clearances as has meat; and the subcutaneous or intravenous administration of glycine, glycylglycine and alanine induces immediate but moderate increases in renal activity, whereas glucose is without effect. It would, therefore, appear that the primary effect of protein upon renal function is a result of the action of the amino acids, or of the hydroxy acids produced in the course of their metabolism, upon glomerular activity.

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THE VALUE OF THE CHEMICAL TITRATION METHOD IN DETERMINING THE VITAMIN C POTENCY OF CERTAIN FOOD SUBSTANCES[1]

N. B. GUERRANT, RUSSEL A. RASMUSSEN AND R. ADAMS DUTCHER
Department of Agricultural and Biological Chemistry, Pennsylvania State College, State College

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Recent investigations have shown that vitamin C and ascorbic acid are identical substances (King and Waugh, '32 a and b; Harris and Ray, '33; Herbert, Hirst, Percival, Reynolds and Smith, '33; Reichstein, Grussner and Oppenauer, '33; Ault, Baird, Charrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey, '33) and that ascorbic acid can be estimated quantitatively by titrating with a standard solution of iodine or a standard solution of 2, 6-dichlorophenol-indophenol.

A review of the literature relative to the chemical estimation of vitamin C in foodstuffs (Tillmans and Hirsch, '32; Wolff, Kekeln and Emmerie, '33; Birch, Harris and Ray, '33; Bessey and King, '33) indicated that the method proposed by Bessey and King is the least affected by interfering substances; consequently, this method was used in the following studies.

The present investigation was conducted with the view of attempting to establish further correlations between chemical titration methods and the usual biological method for the estimation of vitamin C in fruit and vegetable juices.

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[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, December 6, 1934, as technical paper no. 669.

EXPERIMENTAL

The studies herein reported consist of the determination of the ascorbic acid content of several fruit and vegetable juices by the chemical method and the subsequent comparison of some of the results obtained by the conventional biological method of vitamin C assay.

Chemical assay

Bessey and King ('33) have pointed out that lemon juice could be used with reasonable accuracy in the standardization of the dye, while Birch, Harris and Ray ('33) stated that both lemon and orange juice are reliable in this respect.

In these studies, lemon juice was always used in the standardization of the dye solution. The technic used is as follows: The dye solutions were prepared just previous to being used by dissolving 0.1 gm. of the dye in successive portions of warm water, diluting to 200 ml., then cooling and filtering. A 5 ml. aliquot of the freshly prepared and strained lemon juice was titrated with 0.01 normal iodine solution until a permanent bluing of the starch indicator resulted. Ten milliliters of an 8 per cent acetic acid solution were added to a second 5 ml. aliquot of the lemon juice and the dye solution run in from a burette until the end point (pink) was reached. By means of this technic, interfering substances which fade the dye slowly are easily detected when the end point has been reached.

Since 1 ml. of 0.01 normal iodine solution reacts with 0.88 mg. of ascorbic acid, the ascorbic acid equivalent of the dye solution was easily calculated. Other juices were then evaluated as to their ascorbic acid content by titrating against this dye solution of known titer.

For the purpose of reference, the dye solutions used were standardized occasionally against pure ascorbic acid as well as against several different fruit juices. In table 1 are given the results obtained by standardizing some of the dye solution against ascorbic acid, lemon juice, orange juice, and grapefruit juice. The results are expressed as milligrams of ascorbic acid for each milliliter of the dye solution.

The above results indicated that lemon juice and grapefruit juice could be used as standards without introducing serious errors in the technic. Orange juice, however, appeared to be less reliable in this respect. While the juice of only one orange was titrated in direct comparison with a known amount of ascorbic acid on each of the above days, other titrations of orange juice showed about the same irregularities when lemon juice was used as the check standard.

Biological assay

The biological assays were carried out according to the technic of Sherman, LaMer and Campbell ('22). Young guinea pigs weighing from 250 to 300 gm. were used as the experimental animals. These pigs were kept in individual metal cages which were provided with raised screen floors. With the exception of a slight modification of the scorbutic diet, these animals were fed and handled according to the technic of the above authors. The basal scorbutic diet used in these studies contained 2 per cent of cod liver oil (Patch's medicinal grade) and 8 per cent of butter fat instead of the usual 10 per cent of butter fat. This alteration in the composition of the scorbutic diet was made in order to insure a liberal supply of vitamin D.

TABLE 1

Showing the results obtained by standardizing some of the dye solutions against ascorbic acid, lemon juice, orange juice and grapefruit juice

DATE	ASCORBIC ACID STANDARDIZATION	LEMON JUICE STANDARDIZATION	ORANGE JUICE STANDARDIZATION	GRAPEFRUIT STANDARDIZA
4/25/34	0.147[1]	0.155	0.165	....
5/24/34	0.149[2]	0.150	0.160	0.150
6/26/34	0.144[3]	0.152	....	0.146

[1] Natural ascorbic acid furnished through the courtesy of Dr. C. G. King.

[2] Natural ascorbic acid furnished through the courtesy of Merck & Co.

[3] Synthetic ascorbic acid furnished through the courtesy of Hoffmann-LaRoche & Co.

The first series of biological assays for vitamin C were made on fresh Florida orange juice and canned pineapple juice. A week's supply of the fresh fruit and the canned juice was kept in the refrigerator at all times during the test period. The juice of the oranges was extracted just previous to titration and feeding. The canned pineapple juice was opened just before being used.

Shrader and Johnson ('34) and Nelson and Molten ('34) had shown that when fruit juices are kept frozen for long periods of time, there is no appreciable loss of antiscorbutic potency. Consequently, for the second series of biological assays, composite samples of the various juices were preserved in the above manner until the assays could be made. In the case of lemon juice, 5 dozen of each of three commercial grades of lemons were used as the source of juice. One dozen select grapefruits and 1 dozen fresh pineapples were used as sources of these juices. The fruits were pared by hand and the juices pressed out mechanically. The juices were strained and placed in small waxed-cardboard containers, each of which held an ample supply for daily experimentation, and then stored at a temperature of —10°F. The freshly thawed juices were fed to guinea pigs on the basis of the calculated minimum protective dosage as determined by the dye titer. It was hoped that by such a procedure, a uniform supply of the various juices could be had for the entire experimental period, and that results could be obtained which would show whether or not the calculated levels of these juices furnished the desired degree of protection to guinea pigs, and, further, that some information might be obtained concerning a possible variation in the antiscorbutic potency of juices from different grades of lemons. The stored juices were titrated from time to time during the storage period against a standard dye solution. The resultant titers showed that no marked decrease in vitamin C potency took place during the 90-day storage period.

Bessey and King ('33) have shown that 0.5 mg. of ascorbic acid daily per guinea pig prevented scurvy, allowed satisfactory survival, and maintained the experimental animals in a condition of reasonable sensitivity to changes in vitamin intake. Therefore, in these studies the calculations have been based on the assumption that 0.5 mg. of ascorbic acid or its equivalent is the minimum protective dose for the average size experimental guinea pig.

DATA AND DISCUSSION

A condensed summary of the data obtained in these studies is given in the following tables.

Table 2 gives the results obtained in a series of biological assays that were made on canned pineapple juice and fresh orange juice. The table also contains the equivalent ascorbic acid content of the respective quantities of the two juices fed, as determined by the dye titration method. In addition, the theoretical International units of vitamin C are given.

TABLE 2

Showing the results obtained by feeding varying amounts of canned pineapple juice and fresh orange juice to groups of young guinea pigs that were receiving a scurvy-producing diet

ANIMAL GROUP NO.	NUMBER OF ANIMALS CONSIDERED	SOURCE OF VITAMIN C (JUICES)	QUANTITY OF JUICE FED DAILY	TITRATION EQUIVALENT IN MILLIGRAMS OF ASCORBIC ACID	THEORETICAL[1] INTERNATIONAL UNITS	AVERAGE DAILY GAIN IN WEIGHT	SCURVY SCORE
			ml.			*gm.*	
1	6	Pineapple	0	0	0	All died	Severe
2	6	Pineapple	3.0	0.245	5.7	All died	Severe
3	3	Pineapple	4.0	0.352	7.6	0	17
4	4	Pineapple	5.0	0.440	9.5	0.5	8
5	9	Pineapple	6.0	0.528	11.4	1.8	3
6	7	Pineapple	7.0	0.616	13.3	2.4	0–2
7	5	Pineapple	8.0	0.707	15.2	2.7	0
8	8	Pineapple	9.0	0.792	17.1	2.7	0
9	7	Pineapple	10.0	0.880	19.0	2.9	0
10	4	Orange	1.0	0.500	11.0	2.8	0–2
11	9	Orange	1.5	0.750	16.5	3.0	0
12	9	Orange	2.0	1.000	22.0	3.2	0
13	8	Orange	3.0	1.500	33.0	3.2	0
14	6	Orange	4.0	2.000	44.0	3.6	0
15	5	Orange	5.0	2.500	55.0	3.7	0

[1] Calculations based on the average value (1.1 ml. = 0.5 mg. of ascorbic acid) obtained by titrating ten different samples of lemon juice.

The six animals comprising group 1, which served as a negative control, were all dead by the thirty-first day, while those animals comprising group 2 were all dead by the forty-second day. On autopsy, all animals of these two groups were found to exhibit symptoms of severe scurvy. Although the animals of groups 3 and 4 survived the experimental period, they likewise showed symptoms of severe scurvy. The animals of group 5 were somewhat irregular in their responses and, when autopsied, their scurvy score ranged from 1 to 6, with an average score of 3.

The animals of group 6 showed only very mild symptoms of scurvy and their growth rates were not significantly depressed. Due to these facts, this group of animals was considered as having received a minimum protective dose of vitamin C or the equivalent of 0.5 mg. of ascorbic acid daily.

The animals of groups 7, 8 and 9 all showed practically the same growth rate and survived the experimental period without showing any evidence of scurvy.

The animals of group 10, which received orange juice, made a similar response to that of those animals comprising group 6, which received the canned pineapple juice. From this it appeared that 1 ml. of fresh orange juice was equivalent to about 7 ml. of canned pineapple juice in antiscorbutic potency. Results obtained by the dye titration had indicated that the relative antiscorbutic potencies of the two juices would be about 6 : 1.

The animals of groups 11, 12, 13, 14 and 15 showed no evidence of scurvy during the experimental period and when autopsied they appeared to be normal in every respect.

In table 3 are given the results of a second series of biological assays. It may be observed from the data given in this table that the biological responses of the several groups of animals were quite uniform. In every case, with the exception of one group, the calculated dosage gave the expected response. The animals of group 16, which received the calculated sub-protective dosage of fresh pineapple juice, did not show as severe scurvy symptoms as might have been expected from the results given in a previous publication on this subject (Bessey and King, '33).

Table 4 contains the data obtained by titrating the juices of several fruits and vegetables against a standard solution

TABLE 3

Showing the biological responses made by young guinea pigs receiving a scorbutic diet when this diet was supplemented by such quantities of the various fruit juices as would furnish a definite amount of ascorbic acid

ANIMAL GROUP NO.	NUMBER OF ANIMALS CONSIDERED	SOURCE OF VITAMIN C (JUICES)	QUANTITY OF JUICE FED DAILY	TITRATION EQUIVALENT IN MILLIGRAMS OF ASCORBIC ACID	INTERNATIONAL UNITS	AVERAGE DAILY GAIN IN WEIGHT	SCURVY SCORE
			ml.			*gm.*	
16	3	Pineapple	1.0	0.3	6.6	2.4	6
17	5	Pineapple	1.6	0.5	11.0	2.4	0–2
18	5	Pineapple	2.2	0.7	15.4	3.4	0
19	4	Lemon I	1.1	0.5	11.0	2.8	0–2
20	3	Lemon II	1.1	0.5	11.0	2.5	0–2
21	5	Lemon III	1.0	0.5	10.0	2.8	0–2
22	6	Grapefruit	1.9	0.5	11.0	2.7	0–2

TABLE 4

Showing the ascorbic acid equivalent and the calculated minimum protective dose of various fruit and vegetable juices as determined by titrating against a standardized solution of 2, 6-dichlorophenolindophenol

KINDS OF JUICE USED	QUALITY OF JUICE	NUMBER OF SAMPLES CONSIDERED	ASCORBIC ACID EQUIVALENT	CALCULATED MINIMUM PROTECTIVE DOSAGE (AVERAGE)
			(*mg. per ml.*)	(*ml.*)
Lemon	Fresh	13	0.455	1.1
Orange	Fresh	10	0.500	1.0
Orange	Canned	2	0.294	1.7
Tangerine	Fresh	2	0.208	2.4
Lime	Fresh	1	0.459	1.1
Grapefruit	Fresh	1	0.266	1.9
Grapefruit	Canned	1	0.410	1.2
Pineapple	Fresh	2	0.310	1.6
Pineapple	Canned	18	0.070	7.2
Tomato	Fresh	1	0.157	3.2
Tomato	Canned	1	0.242	2.1
Peach	Fresh	1	0.018	27.8
Plum	Fresh	1	0.030	16.7
Rhubarb	Fresh	1	0.278	1.8
Sauerkraut	Canned	1	0.082	6.2
Cabbage	Fresh	1	0.427	1.2
Peppers	Green	1	0.203	2.5
Carrots	Fresh	1	0.040	12.5
Celery	Fresh	1	0.009	55.6
Turnip	Fresh	1	0.191	2.6
Cucumber	Fresh	1	0.012	41.6
Potato	Fresh	1	0.021	23.8

of the dye. In this table, the results are expressed in terms of the ascorbic acid equivalent in milligrams per milliliter of juice and as the quantity required to furnish a minimum protective dose.

SUMMARY

A study has been made concerning the value of the chemical titration method for determining the vitamin C potency of certain food substances. In these studies, the ascorbic acid content of certain fruit and vegetable juices was determined by titrating against a standard solution of 2, 6-dichlorophenol-indophenol. In order to ascertain the accuracy of the data obtained through the chemical titration method, biological assays were also made on a number of the fruit juices used in this study. From the results obtained it appears that:

1. The chemical titration method for the estimation of ascorbic acid is of material value in determining the vitamin C potency of certain fruit and vegetable juices. By means of this method, it is possible at least to approximate the antiscorbutic activity of the juices before the biological assay is started, thus saving time and expense.
2. The accuracy of the results obtained by the chemical titration method appears to depend to an appreciable degree upon the nature of the juice that is being titrated. Some juices contain interfering substances that react with the dye, thus complicating the titration results and leading to erroneous conclusions.
3. For lemon juice, orange juice, grapefruit juice and fresh pineapple juice, the values obtained by the chemical titration method are in close agreement with those obtained by the usual biological assay method.
4. Fresh pineapple juice, when evaluated for its antiscorbutic potency by the chemical method, gives values in closer agreement with those obtained by the biological method than does the canned pineapple juice, owing to the presence of interfering substances in the latter.

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UTILIZATION OF MEAT BY HUMAN SUBJECTS[1]

II. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF ROUND AND LIVER OF BEEF

ZELDABETH LONG AND MARTHA S. PITTMAN
Kansas Agricultural Experiment Station, Manhattan

(Received for publication January 7, 1935)

Mitchell ('27) has suggested that the nitrogen of beef liver has a higher biological value for rats than that of beef round, attributing the difference to some extent to relatively larger amounts of connective tissue in the round. The study here reported was made to see if similar results for nitrogen would be obtained with human subjects and to determine the utilization of the phosphorus from the two meats. Samples were also analyzed for collagen and elastin nitrogen, so the relationship of these factors to the utilization of total nitrogen and phosphorus might be studied.

The subjects were three healthy young college women, ranging in weight from 56 to 60 kg., with activities varying from light to moderate exercise. Subjects A and B consumed 39 and subject C 42 Calories per kilogram daily, amounts sufficient to maintain their weights.

The experiment was divided into two parts, one for each meat. Each part consisted of a 4-day preliminary period followed by two 4-day experimental periods. The meats[2] were of good grade and of known history. Equal weights of liver and round were used from the same animal, thus eliminating differences due to grade. Each meat was freed from visible fat, coarsely ground once to permit of sampling, and mixed well. Weighed portions for every lunch and dinner for each individual were wrapped separately and kept frozen until used. Then they were browned without fat and cooked slowly in individual pans in a small amount of distilled water.

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[1] Contribution no. 37, Department of Home Economics.

[2] The authors wish to thank Prof. David Mackintosh, of the Department of Animal Husbandry of Kansas State College, for supplying the meats.

The diet (table 1) consisted of the meat to be tested, a low-nitrogen bread of cracker-like texture, filtered butter fat,

TABLE 1

Daily food intake

SUBJECT	FOOD	BEEF ROUND PERIOD				BEEF LIVER PERIOD			
		Weight	Protein	Phosphorus	Calories	Weight	Protein	Phosphorus	Calories
		gm.	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	
A and B (56 kg. each)	Meat	131.0	28.81	0.232	204	136.8	30.88	0.493	176
	Bread [1]		0.53		927		0.53		927
	Butterfat, filtered	17.0			153	20.5			185
	Beverage [2]	1120.0	11.75	0.188	910	1120.0	8.75	0.179	910
	Calcium carbonate	0.550				0.538			
	Ferric citrate	0.043				0.008			
	Sodium chloride	3.000				3.000			
	Total		41.09	0.420	2194		40.16	0.672	2198
C (60 kg.)	Meat	143.2	31.63	0.253	223	149.5	33.69	0.539	193
	Bread [1]		0.53		927		0.53		927
	Butterfat, filtered	15.0			135	18.5			167
	Beverage [2]	1120.0	11.75	0.188	910	1120.0	8.75	0.179	910
	Candy [3]				321				321
	Calcium carbonate	0.680				0.643			
	Ferric citrate	0.042				0.004			
	Sodium chloride	3.000				3.000			
	Total		43.91	0.441	2516		42.97	0.718	2518

[1] Bread: cornstarch, 120 gm.; lactose, 40 gm.; Dextri-maltose, 15 gm.; baking powder, tartrate, 3 gm.; sodium chloride, 3 gm.; Agar, 4 gm.; butterfat, filtered, 32 gm.; water, distilled, 80 gm.

[2] Beverage: orange juice, 1000 gm.; lactose, 80 gm.; sucrose, 40 gm.

[3] Candy: sucrose, 70 gm.; cornstarch, 4 gm.; butterfat, filtered, 3 gm.; water, distilled, 60 gm.

and orange juice with lactose and sucrose. Subject C also ate a fondant-like candy. During the liver period the butter fat was increased slightly to maintain the caloric intake; otherwise the two diets were the same, except for the meat. Both diets were base-forming. Distilled water was used in approximately constant amounts by each subject, ranging from 650 to 1000 cc. daily, according to individual preferences. The sodium chloride content of the diet was also controlled. Calcium and iron were brought up to accepted standards (Sherman, '32) by addition of calcium carbonate and ferric citrate.

Analysis of the foods used showed the meats to be somewhat higher in nitrogen and the orange juice and cornstarch much higher than expected. This placed the nitrogen somewhat above the calculated level of 85 per cent of the protein requirement suggested by Sherman ('32), i.e., 44.4 gm. per 70 kg. of body weight, and reduced the percentage supplied by the meats to an average of approximately 71 per cent of the total nitrogen for the beef round period and to 77 per cent for the beef liver. However, since the protein level was still low and the proportion supplied by the meat high, it is believed the results obtained may be attributed largely to the meat consumed.

The round supplied an average of 56 per cent of the total dietary phosphorus and the liver 74 per cent—amounts lower than desired, but still providing the major portion of the phosphorus in the diet. On the basis of 0.88 gm. phosphorus daily per 70 kg. of body weight (Sherman, '32), the phosphorus intake in the beef round period averaged only 59 per cent of the amount needed by the different subjects, whereas when liver was eaten, the requirement was somewhat more than met.

Urine was collected quantitatively for each 24-hour day and feces for each 4-day period. The usual precautions were used in caring for samples. Total nitrogen was determined by the Kjeldahl-Gunning procedure; phosphorus by the volumetric method of the Association of Official Agricultural Chemists;

and collagen and elastin nitrogen by a revised method from the Illinois Agricultural Experiment Station.

All subjects were in positive nitrogen balance with both meats (table 2). With an average daily intake of approximately 0.12 and 0.11 gm. nitrogen per kilogram, respectively, for the two meats, the average daily retention for the 8 experimental days of the beef round period was + 0.91 ± 0.11 and for the beef liver + 0.82 ± 0.11 gm. per person or + 0.016 and + 0.014 gm. per kilogram. For the 24 experimental days on each meat for the entire group, there were twenty-two positive balances for round and twenty-one for liver. Two subjects had negative balances in each case.

The average coefficients of digestibility for the nitrogen for the various subjects on beef round ranged from 79.3 to 84.8 per cent and averaged 82.5 per cent for all subjects. With beef liver it ranged from 73.7 to 86.6 per cent and averaged 82.1 per cent, which was practically the same as for round. Subjects B and C had higher coefficients of digestibility for liver nitrogen, but subject A digested round better. The lowest values for both meats were for subject A indicating that individual differences are an important factor. Although the diet contained more nitrogen when round was eaten, no difference in urinary nitrogen output was observed. It averaged 0.08 gm. per kilogram of body weight per day for each kind of meat; again suggesting that the nitrogen of the two meats was used about equally well. Any differences observed apparently were not significant.

Both collagen and elastin nitrogen were lower in liver than in round of beef (table 2). Since any difference in utilization of these two meats was slightly in favor of beef round, something other than connective tissue must have influenced the results.

The average phosphorus balance for all subjects for both meats was slightly negative amounting to — 0.035 ± 0.015 gm. per person per day for the round and — 0.010 gm. ± 0.020 gm. for the liver which, in the latter case, was approximate equilibrium. Two subjects were in negative phosphorus

TABLE 2

Average daily nitrogen and phosphorus balances

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE		
		Meat	Total	Urine	Feces	Total	Total	Per kg.	Per 70 kg.
Nitrogen[1]									
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A (56 kg.)	Round[2]	4.61	6.57	4.11	1.36	5.47	+ 1.10 ± 0.13	+ 0.020	+ 1.40
	Liver[3]	4.94	6.42	4.27	1.69	5.96	+ 0.46 ± 0.26	+ 0.008	+ 0.56
B (56 kg.)	Round	4.61	6.57	4.84	1.10	5.94	+ 0.63 ± 0.22	+ 0.011	+ 0.77
	Liver	4.94	6.42	4.48	0.89	5.37	+ 1.05 ± 0.17	+ 0.019	+ 1.33
C (60 kg.)	Round	5.06	7.03	4.94	1.07	6.01	+ 1.02 ± 0.25	+ 0.017	+ 1.19
	Liver	5.39	6.88	5.01	0.92	5.93	+ 0.95 ± 0.12	+ 0.016	+ 1.12
Average for all subjects (57.3 kg.)	Round	4.76	6.72	4.63	1.18	5.81	+ 0.91 ± 0.11	+ 0.016	+ 1.12
	Liver	5.09	6.57	4.59	1.17	5.75	+ 0.82 ± 0.11	+ 0.014	+ 0.98
Phosphorus[1]									
								mg.	
A (56 kg.)	Round	0.232	0.420	0.242	0.175	0.417	+ 0.003 ± 0.007	+ 0.05	+ 0.004
	Liver	0.493	0.672	0.499	0.224	0.723	— 0.051 ± 0.060	— 0.91	— 0.064
B (56 kg.)	Round	0.232	0.420	0.277	0.199	0.476	— 0.056 ± 0.012	— 1.00	— 0.070
	Liver	0.493	0.672	0.441	0.219	0.660	+ 0.012 ± 0.027	+ 0.21	+ 0.015
C (60 kg.)	Round	0.253	0.441	0.262	0.230	0.492	— 0.051 ± 0.046	— 0.85	— 0.060
	Liver	0.539	0.717	0.438	0.269	0.707	+ 0.010 ± 0.024	+ 0.17	+ 0.012
Average for all subjects (57.3 kg.)	Round	0.239	0.427	0.260	0.201	0.462	— 0.035 ± 0.015	— 0.60	— 0.042
	Liver	0.508	0.687	0.459	0.237	0.697	— 0.010 ± 0.020	— 0.18	— 0.013

[1] Average of 8 experimental days.

[2] Collagen furnished 6.69 per cent of total nitrogen; elastin 0.29 per cent.

[3] Collagen furnished 3.07 per cent of total nitrogen; elastin 0.20 per cent.

(Analyses for collagen and elastin were made by the Chemistry Laboratory of the Kansas Agricultural Experiment Station.)

balance on round, whereas only one was negative on liver, but when round was eaten the diet averaged 7.4 mg. phosphorus per kilogram of body weight; in the liver period, 12.0 mg. per kilogram. Both fecal and urinary phosphorus were higher for all subjects when liver was eaten, as was to be expected with the higher intake in this period. However, fecal phosphorus was lowered from an average of 43.5 per cent for round to 34.0 per cent for liver, which suggests superior absorption of liver phosphorus.

The large amounts of orange juice and lactose in the diet probably favored the use of the phosphorus, since they are believed to increase acidity in the digestive tract and raise absorption accordingly (Bergeim, '26; Chaney and Blunt, '25). The basic reaction of the foods used may also have had an effect. On the other hand, the diet was low in vitamin D and the ultraviolet light received by the subjects was limited—factors unfavorable to best usage of phosphorus—as was a probable disproportion in the ratio of phosphorus to calcium.

SUMMARY

Three young women apparently used the nitrogen of round of beef fully as well as that from liver of beef, as indicated by almost identical coefficients of digestibility and a slightly more favorable nitrogen balance during the period round was eaten. The latter may be accounted for by a slightly higher intake.

The phosphorus balance was but little lower on round, although the diet during this period supplied less than two-thirds as much phosphorus. A higher percentage of the liver phosphorus may have been absorbed.

The collagen and elastin were higher in round than in liver, so apparently could not have affected seriously the utilization of the total nitrogen of this cut.

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UTILIZATION OF MEAT BY HUMAN SUBJECTS[1]

III. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF BEEF HEART

BERNICE L. KUNERTH, IDA M. CHITWOOD AND MARTHA S. PITTMAN
Kansas Agricultural Experiment Station, Manhattan

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Beef heart has been reported (Mitchell, '27) to have a biological value for rats somewhat lower than liver, but superior to round. To investigate the value of beef heart for human subjects, the utilization of its nitrogen and phosphorus has been studied by means of balance experiments. Beef round was used as a control. The subjects, four healthy young college women, maintained their weights (table 2) throughout the experiment on an intake of 45 Calories per kilogram.

The diet (table 1) and procedure for conducting the experiment were essentially the same as in earlier studies (Long and Pittman, '35). A good grade of beef of known origin was used. The heart and an equal weight of round were taken from each of eleven carcasses. The meats[2] were prepared, stored, and cooked as previously described.

The diet furnished approximately the daily protein requirement of 44.4 gm. per 70 kg. (Sherman, '32) for the beef round period and from 13 to 18 per cent in excess of this during the beef heart period. The daily phosphorus intake averaged 67 per cent of the requirement of 0.88 gm. per 70 kg. (Sherman, '32) for the beef round period and 88 per cent for the

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[1] Contribution no. 42, Department of Home Economics.

[2] The authors wish to thank Prof. David Mackintosh, of the Department of Animal Husbandry of Kansas State College, for supplying the meats.

TABLE 1

Daily food intake

SUBJECT	FOOD	BEEF ROUND PERIOD				BEEF HEART PERIOD			
		Weight	Protein	Phosphorus	Calories	Weight	Protein	Phosphorus	Calories
		gm.	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	
A	Meat	113.6	21.9	0.203	132	151.4	24.9	0.319	176
	Bread[1]		0.4		927		0.4		927
	Butterfat, filtered	20.0			180	15.0			135
	Beverage[2]	1120.0	9.5	0.205	910	1120.0	10.0	0.216	910
	Calcium carbonate	0.400				0.399			
	Ferric citrate	0.024				0.020			
	Sodium chloride	4.000				4.000			
	Total		31.8	0.408	2149		35.3	0.535	2148
B	Meat	125.6	24.1	0.225	146	167.3	27.5	0.353	194
	Bread[1]		0.4		927		0.4		927
	Butterfat, filtered	43.2			389	38.0			342
	Beverage[2]	1120.0	9.5	0.206	910	1120.0	10.0	0.217	910
	Calcium carbonate	0.518				0.517			
	Ferric citrate	0.028				0.023			
	Sodium chloride	4.000				4.000			
	Total		34.0	0.431	2372		37.9	0.570	2373
C	Meat	127.8	24.4	0.229	148	170.0	28.1	0.359	197
	Bread		0.4		927		0.4		927
	Butterfat, filtered	47.7			429	42.0			378
	Beverage	1120.0	9.5	0.205	910	1120.0	10.0	0.215	910
	Calcium carbonate	0.539				0.538			
	Ferric citrate	0.028				0.024			
	Sodium chloride	4.000				4.000			
	Total		34.3	0.434	2414		38.5	0.574	2412
D	Meat	131.5	25.0	0.236	153	175.2	28.8	0.369	203
	Bread		0.4		927		0.4		927
	Butterfat, filtered	55.0			495	49.3			444
	Beverage	1120.0	9.5	0.206	910	1120.0	10.0	0.217	910
	Calcium carbonate	0.577				0.585			
	Ferric citrate	0.029				0.025			
	Sodium chloride	4.000				4.000			
	Psyllium seed	18.000	2.1	0.068		18.000	2.2	0.068	
	Total		37.0	0.510	2485		41.4	0.654	2484

[1] Bread: cornstarch, 120 gm.; lactose, 40 gm.; dextri-maltose, 15 gm.; baking powder, tartrate, 3 gm.; sodium chloride, 3 gm.; agar, 5 gm.; butterfat, filtered, 32 gm.; water, distilled, 80 gm.

[2] Beverage: orange juice, 1000 gm.; lactose, 80 gm.; sucrose, 40 gm.

beef heart period. The unexpectedly high nitrogen content of the orange juice and the difference in the amount of nitrogen in the meats from the calculated values made the nitrogen level of the diet higher than was desired and reduced the proportion supplied by the meats. The meats actually furnished an average of only 70 per cent of the total nitrogen and 50 per cent of the total phosphorus for all subjects in the beef round period and 71 per cent of the total nitrogen and 60 per cent of the total phosphorus when beef heart was eaten.

The methods of collection, preservation, and analysis were the same as described by Pittman ('32).

The average nitrogen balance for all subjects was slightly positive for both meats, amounting to $+ 0.02 \pm 0.12$ gm. for round and $+ 0.60 \pm 0.10$ gm. for heart. The difference between the values for the two meats is of doubtful significance ($\frac{0.58}{0.16} = 3.6$) and may be due to the level at which the meats were fed rather than to quality of nitrogen. Three subjects showed a negative balance for the 8 days of the round period on an intake of 0.11 gm. nitrogen per kilogram, whereas all were in positive balance when heart was eaten at the somewhat higher level of 0.12 gm. per kilogram. There were fifteen positive balances for round and twenty-seven for heart out of 32 and 31 experimental days, respectively. Long and Pittman ('35) obtained, with beef round on a similar diet, an average balance of $+ 0.91 \pm 0.11$ gm. with an intake of 0.12 gm. nitrogen per kilogram. The average coefficients of digestibility for nitrogen in the present study were practically identical for the two meats approximating 87 per cent for both. As noted in other experiments, variations in the same subject from day to day and individual differences were greater than those noted between meats.

Urinary nitrogen averaged 0.089 gm. per kilogram daily for all subjects with each cut of meat and, as coefficients of digestibility were also similar, it appears that retention of nitrogen from the two meats was equally good, though the round supplied a smaller amount.

The heart contained more than twice as much collagen[3] as the round and but little more elastin (table 2). As heart showed somewhat higher balances for both nitrogen and phosphorus than round and was equally well digested, its larger amount of connective tissue apparently did not decrease its value to a noticeable degree. This beef round was approximately 25 per cent higher in both forms of connective tissue than that used by Long and Pittman ('35) which may help to explain the higher balance they obtained.

The average daily phosphorus balance for all subjects on beef round with an intake averaging 8.5 mg. per kilogram was -0.054 ± 0.009 gm. During the heart period, with intake averaging 11.1 mg. per kilogram, the average daily balance was more favorable, amounting to $+0.002 \pm 0.010$ gm. While the difference between the two values may appear to be significant ($\frac{0.056}{0.009} = 6$), it is probable that it was also due to the level at which the meats were fed. All subjects were in negative phosphorus balance on beef round; with heart, as was to be expected with a higher intake, all approached equilibrium, two being slightly negative and two slightly positive. Superior quality of meat phosphorus is suggested when it is compared with that from navy beans (Pittman, '32) where, with an intake slightly higher per unit of weight than the highest for meat, the balance was considerably more negative. The results reported by Long and Pittman ('35) showed a slightly more favorable usage of beef round phosphorus on a somewhat lower intake per kilogram than was obtained in these experiments. Their values for beef liver, on a somewhat higher intake of phosphorus per unit of weight, were close to these for beef heart.

With one exception, both urinary and fecal phosphorus were increased in the beef heart period, though the percentage distribution did not change greatly. It appears that absorption of phosphorus from the two meats was not widely different.

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[3] Analyses were made by the Chemistry Laboratories of the Kansas Agricultural Experiment Station.

TABLE 2

Average daily nitrogen and phosphorus balances

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE		
		Meat	Total	Urine	Feces	Total	Total	Per kg.	Per 70 kg.
					Nitrogen [1]				
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A	Round [2]	3.49	5.06	4.64	0.64	5.28	− 0.22 ± 0.26	− 0.005	− 0.35
(47 kg.)	Heart [3]	3.98	5.65	4.16	0.93	5.09	+ 0.56 ± 0.11	+ 0.012	+ 0.84
B	Round	3.86	5.43	5.03	0.74	5.77	− 0.34 ± 0.33	− 0.006	− 0.42
(52.7 kg.)	Heart	4.40	6.05	4.58	0.60	5.18	+ 0.87 ± 0.28	+ 0.016	+ 1.12
C	Round	3.92	5.50	4.94	0.61	5.55	− 0.05 ± 0.07	− 0.001	− 0.07
(53.6 kg.)	Heart	4.47	6.14	4.98	0.83	5.81	+ 0.33 ± 0.11	+ 0.006	+ 0.42
D [4]	Round	4.04	5.96	4.13	1.14	5.27	+ 0.69 ± 0.18	+ 0.012	+ 0.84
(55.2 kg.)	Heart	4.61	6.63	4.91	1.03	5.94	+ 0.69 ± 0.08	+ 0.012	+ 0.84
All subjects	Round	3.83	5.49	4.63	0.78	5.41	+ 0.02 ± 0.12	± 0.000	± 0.00
(52.3 kg.)	Heart	4.36	6.12	4.65	0.86	5.52	+ 0.60 ± 0.10	+ 0.011	+0.77
					Phosphorus [1]				
								mg.	
A	Round	0.203	0.408	0.315	0.168	0.483	− 0.075 ± 0.010	− 1.57	− 0.110
(47.7 kg.)	Heart	0.319	0.535	0.339	0.205	0.544	− 0.009 ± 0.019	− 0.19	− 0.013
B	Round	0.225	0.430	0.304	0.215	0.519	− 0.089 ± 0.025	− 1.69	− 0.118
(52.7 kg.)	Heart	0.353	0.568	0.379	0.178	0.557	+ 0.011 ± 0.032	+ 0.21	+ 0.015
C	Round	0.229	0.434	0.293	0.167	0.460	− 0.026 ± 0.009	− 0.49	− 0.034
(53.2 kg.)	Heart	0.359	0.574	0.380	0.210	0.590	− 0.016 ± 0.016	− 0.30	− 0.021
D [4]	Round	0.236	0.509	0.316	0.219	0.535	− 0.026 ± 0.017	− 0.47	− 0.033
(55.2 kg.)	Heart	0.369	0.652	0.403	0.226	0.629	+ 0.023 ± 0.010	+ 0.42	+ 0.029
All subjects	Round	0.223	0.445	0.307	0.192	0.499	− 0.054 ± 0.009	− 1.03	− 0.072
(52.3 kg.)	Heart	0.350	0.582	0.375	0.205	0.580	+ 0.002 ± 0.010	+ 0.04	+ 0.003

[1] Average of 8 experimental days, except for subject B in beef heart period, when 7 days were used.

[2] Collagen furnished 8.58 per cent of total nitrogen; elastin 0.36 per cent.

[3] Collagen furnished 18.90 per cent of total nitrogen; elastin 0.39 per cent.

[4] Daily diet included 0.35 gm. nitrogen and 0.068 gm. phosphorus from psyllium seed.

SUMMARY

Four college women seemingly used the nitrogen of beef heart at least as well as that from beef round as indicated by practically identical coefficients of digestibility and losses of urinary nitrogen. A higher nitrogen balance for heart may probably be explained by a somewhat higher nitrogen intake.

Differences in utilization of phosphorus from the two meats, which were also in favor of beef heart, were not great and probably were also due to level in the diet. The heart contained more connective tissue than the round, but, as the balances for both nitrogen and phosphorus were slightly higher for this meat, it apparently did not prevent good usage of these elements.

Daily variations in the nitrogen and phosphorus metabolism of an individual subject and differences between subjects were often greater than the differences in availability of these elements from the two meats.

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THE NUTRITIVE VALUE OF FUNGI

II. THE VITAMIN B, G AND B_4 CONTENT OF THE MYCELIUM OF ASPERGILLUS SYDOWI[1]

H. J. GORCICA, W. H. PETERSON AND H. STEENBOCK
Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin, Madison

FOUR FIGURES

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Although yeast has been widely used for several decades as a source of vitamin B, the closely related filamentous fungi belonging to the genera Aspergillus and Penicillium have not been examined for this substance until very recently.

Takata ('29) tested the dried mycelium of A. oryzae as a source of vitamin B for young growing albino rats. He concluded that the dried mycelium had about one-fifth the amount of the growth-promoting vitamin B possessed by dried brewer's yeast. Takahashi ('29) grew A. oryzae through many transfers on a modified Pfeffer's solution to exhaust all the vitamin B, and then grew the mold in larger quantities on the same medium. An extract of the mold caused good growth of pigeons and rats, and protected them from polyneuritis. Fawns and Jung ('33) reported that rats were cured of beriberi when a black mold developed spontaneously on their basal vitamin B-free diet. When no mold had formed on the basal diet the animals lost weight.

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[1] The authors are indebted to Professors Hart and Elvehjem for advice regarding the technic which they have developed in the use of the chick for vitamins B, G and B_4 assay. They are also indebted to Dr. O. L. Kline for assistance in carrying out certain phases of the assay.

This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

In a previous report from this laboratory (Skinner, Peterson and Steenbock, '33) it was shown that the dried mycelium of A. fischeri and A. sydowi, when fed at a 20 per cent level, contained sufficient vitamin B to promote good growth of young rats and to afford complete protection against polyneuritis. Animals fed at a 5 per cent level failed to grow, and eventually became polyneuritic.

The present work was first undertaken to determine more accurately the content of the vitamin B complex in mold mycelium. Later, it was decided to determine as well the presence of various members of the vitamin B complex, availing ourselves of the use of the chick as the experimental animal.

EXPERIMENTAL

Preparation of mold mycelium. The mold used in these experiments was Aspergillus sydowi, one of the species used in our previous work (Skinner, Peterson and Steenbock, '33). It was grown under aseptic conditions on a sterilized medium of the following composition: 10 gm. NH_4NO_3, 6.8 gm. KH_2PO_4, 5 gm. $MgSO_4.7H_2O$, 0.16 gm. $FeCl_3.6H_2O$, 0.05 gm. $ZnSO_4.7H_2O$, and 100 gm. glucose, made up to 1 liter with tap water (Peterson, Pruess, Gorcica and Greene, '33). On this medium any vitamin found in the mycelium may be considered as having been synthesized by the organism. The mold was allowed to grow for 10 days. The mold mycelium was then removed, washed with cold water under the tap, squeezed lightly by hand to free it from water, packed into cans, frozen and stored at a temperature of 5°F. It was removed from cold storage as needed, ground to a fine paste and incorporated wet into the ration.

The rations for both rats and chickens, made up in quantities for 1 week's feeding, were kept in tightly-closed containers which were stored in an electric refrigerator. Because of the fact that growth of mold often took place on the mixed rations left in the cages overnight, especially on those with a high moisture content, the unconsumed portions were generally discarded each morning.

Composition of rat rations. The basal ration used with rats had the following composition: cooked cornstarch, 76 parts; acid-extracted casein, 18 parts; salt mixture no. 40 (Steenbock and Nelson, '23), 4 parts; and cod liver oil, 2 parts. Mold was added to this at levels of 10 and 20 per cent calculated on the dried basis, the amount of starch being reduced correspondingly. The ration therefore varied in water content according to the amount of mold added. The mold itself had a fairly constant water content of 75 per cent, with the exception of the batch which was fed for the first 3 weeks to the group getting mold at a 20 per cent level.

Mold as a source of the vitamin B complex for rats. Young male rats weighing from 50 to 60 gm. were used in this experiment. Each rat was placed in an individual cage with a screen bottom and screen sides. Each animal had his own feed cup in which a weighed portion of ration was placed each day, after any uneaten portion was discarded.

The group getting 20 per cent mold as the sole source of vitamin B was used as a control in another experiment and was limited to an intake of 537 gm. (dry basis) per rat over the 10 weeks period. This represents 107 gm. of dry mold eaten during the experiment, or an average intake of 1.53 gm. of mold per rat per day.

The growth curve (average of six) of this group is given in figure 1. The growth secured was excellent, the average gain of the group in 10 weeks was 139 gm. The break in the first part of the curve is due to limited consumption because of the high water content of the ration during the second week. The animals appeared to be in the best of health during the experiment. The portions of ration weighed out in the cups were completely consumed by each animal throughout the experiment, and the animals always seemed exceedingly hungry. There is little doubt that the animals would have eaten considerably more food if it had been given to them. The growth curve of this group represents the efficiency with which the ration was utilized rather than the greatest possible growth on the ration.

The group ate 96.7 gm. of casein and 38.7 gm. mold protein,[2] or 135.4 gm. of total protein. Thus the average gain in weight represents a gain of 1.03 gm. per gram of total protein consumed.

The group getting 10 per cent mold as sole source of vitamin B was not limited in consumption, except in so far as was necessary to keep the intake of the entire group approximately equal. The total intake in 10 weeks was 522 gm. The group made an average gain in weight of 130 gm. The consumption of mold was 52.2 gm. (dry basis) per rat in 10 weeks, or an average of 0.75 gm. per rat per day. Toward the end of the period three of the six rats showed a slightly decreased consumption of food. Except for this slight loss in appetite, none of the animals gave any signs of paralysis or any other disorder.

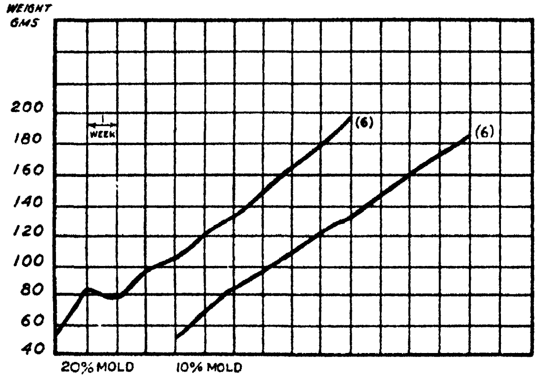

Fig. 1 Mold mycelium as a source of the vitamin B 'complex' for rats. The figures in parentheses indicate the number of individuals represented in each curve.

From the results obtained with these two groups, we may conclude that 20 per cent of mold (dry basis) in the ration, yielding 1.5 gm. of mold daily, furnished enough vitamin B to support good growth in young rats. Mold fed at a 10 per cent level yielding 0.75 gm. per day was almost as effective in promoting growth, but did not supply quite as great a stimulus to the appetite.

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[2] Mold protein as calculated from N × 6.25. This is known to be too high a figure, since much of the mold nitrogen is non-protein in character (Gorcica, Peterson and Steenbock, '34).

Consumption of chick rations. The technic and the rations used in demonstrating the presence of various members of the vitamin B complex were those worked out by members of this department (Kline, Keenan, Elvehjem and Hart, '32; Keenan, Kline, Elvehjem, Hart and Halpin, '33).

The basal ration used for the determination of vitamin B (B_1) contained the following: yellow corn, 58 parts; wheat middlings, 25 parts; casein, 12 parts; NaCl, 1 part; $CaCO_3$, 2 parts; cod liver oil, 2 parts. This ration, however, contains plenty of vitamin B, because it supports normal growth; on it the chicks may attain a weight of 300 gm. at the end of 6 weeks. To make it deficient in vitamin B, the yellow corn, wheat middlings, casein and salt were autoclaved at 120° and 15 pounds pressure for 5 hours. Four per cent of yeast which had been autoclaved for 5 hours at 15 pounds pressure was then added to supply an adequate amount of B_2(G), since the amount of G in the mold was not known. The ration treated in this manner has been designated as ration 241A.

A basal ration suitable for testing for vitamin G was prepared by heating the yellow corn, wheat middlings, casein and salt of the above basal ration at 100° for 144 hours in the dry state. This ration has been designated as ration 240H.

Ration 441A was used as the basal ration for determining vitamin B_4. This ration had the following composition: cooked cornstarch, 53.5 parts; casein autoclaved 5 hours at 15 pounds, 24.0 parts; salts, 2.5 parts; yeast, 8.0 parts; liver residue autoclaved 10 hours at 15 pounds, 10.0 parts; cod liver oil, 2 parts. Chicks on this ration develop a paralysis typical of avitaminosis B_4, which can be prevented by dried liver added at an 18 per cent level.

To introduce the mold into the basal rations, the frozen mold was ground to a fine paste, then mixed in while still very cold. The complete rations were packed into containers with tightly-fitting lids and stored in a refrigerator. They were made up fresh each week.

The chicks used for the experiment were day-old White Leghorns with an initial weight of 30 to 35 gm. obtained from the

University Poultry Department. They were placed in groups of ten in cages with screen bottoms. At the end of the first week, the number was reduced to seven in a group.

Mold as a source of vitamin B for chicks. On the basal autoclaved ration (241A) all of the chicks developed polyneuritis in 7 to 9 days and died in from 8 to 12 days (fig. 2). On the basal ration supplemented with 5 per cent mold, all of the chicks developed polyneuritis in from 10 to 16 days and died in from 11 to 19 days.

When mold was added at a level of 10 per cent, only one chick in the group developed polyneuritis. The six survivors grew throughout the period, attaining an average weight of 218 gm. at the end of 6 weeks. This is not normal growth.

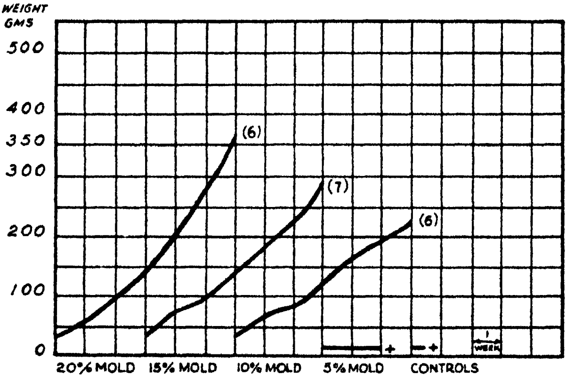

Fig. 2 Mold mycelium as a source of vitamin B for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The heavy horizontal lines followed by crosses indicate the length of life of the chicks on the rations concerned.

At a level of 15 per cent the mold completely prevented the appearance of polyneuritis. The average weight of the group at the end of 6 weeks was 296 gm. At a level of 20 per cent the mold completely prevented polyneuritis and in addition promoted better growth than at any of the lower levels. The chicks in this group attained an average weight of 362 gm. in 6 weeks, which is considered normal for this age.

Thus it is seen that, while 10 per cent of mold in the ration is sufficient to prevent polyneuritis, higher levels improve the growth performance.

Mold as a source of vitamin G for chicks. The growth curves of the chicks in this experiment are shown in figure 3. The chicks in the negative control group (ration 240H) all developed pellagra in 2 to 4 weeks. The most obvious symptoms were the large black ulcers at the corners of the mouth, and, somewhat later, the appearance of a sticky exudate about the eyes. In many cases the eyelids adhered to one another and remained completely closed. At the end of 5 weeks all of the chicks still alive had the above severe symptoms of pellagra and were therefore discarded.

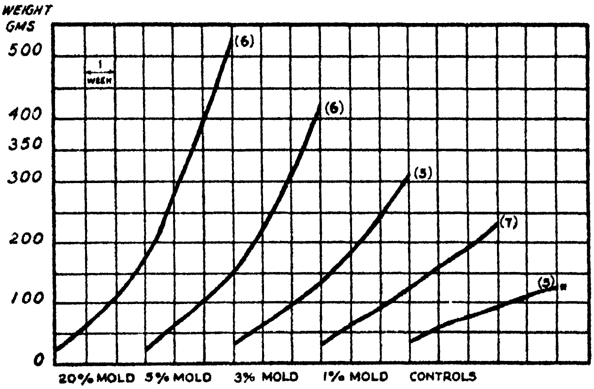

Fig. 3 Mold mycelium as a source of vitamin G for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The star at the end of the curve indicates that all members of the group had severe pellagra and were therefore discarded at that point.

The addition of mold at a 20 per cent level caused an exceptionally rapid growth—the group reached an average weight of 528 gm. in 6 weeks. When 5 per cent of mold was added to the basal ration, the chicks grew very well, reaching an average weight of 417 gm. in 6 weeks. They did not develop any symptoms of pellagra. When the level of mold was reduced to 3 per cent the chicks still grew fairly well, reaching an average weight of 306 gm. in 6 weeks. This level of mold still furnished complete protection against pellagra. At a 1 per cent level the mold still gave the chicks complete protection against pellagra, but the ration no longer supported good growth.

Mold as a source of vitamin B_4 for chicks. The growth curve of the group of chicks serving as negative controls on the basal ration is shown in figure 4. This group did not behave as chicks on ration 441A usually do, and only two of the chicks developed paralysis. This may be attributed to the fact that an error was made in preparing one of the

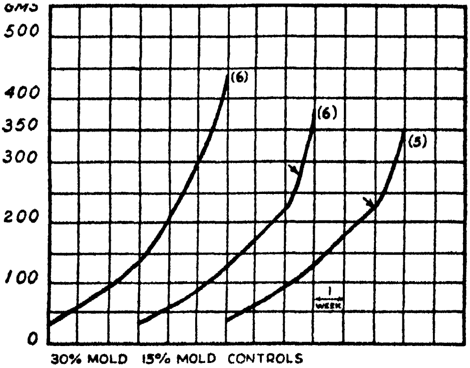

Fig. 4 Mold mycelium as a source of vitamin B_4 for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The arrows indicate the time of appearance of severe symptoms of paralysis.

TABLE 1

Summary

GROUP	RATION	AVERAGE WEEKLY WEIGHT OF CHICKS IN EACH GROUP							
		Start	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Number of survivors
1	R241A (low B)	33	All dead of polyneuritis in 12 days						
2	R241A + 5% mold	35	43	45	All dead of polyneuritis in 19 days				
3	R241A + 10% mold	35	63	79	116	163	190	218	6
4	R241A + 15% mold	35	69	95	140	183	219	296	7
5	R241A + 20% mold	31	56	98	143	201	271	362	6
6	R240H (low G)	33	55	69	90	108	123	—[1]	5
7	R240H + 1% mold	35	61	89	129	156	181	226	7
8	R240H + 3% mold	35	67	94	135	182	246	306	5
9	R240H + 5% mold	35	63	97	151	217	308	417	6
10	R240H + 20% mold	34	60	105	173	276	389	528	6
11	R441A (low B_4)	35	56	90	136	181	267	351	5
12	R441A + 15% mold	34	53	79	119	166	266	383	6
13	R441A + 30% mold	34	55	91	140	220	312	419	6

[1] All the chicks in the group showed severe symptoms of pellagra and were therefore discarded.

batches of ration. During the third week of the experiment the basal ration was made up with 10 per cent liver that had been autoclaved only half the usual time.

When the basal ration was supplemented by 15 per cent mold, the growth was slightly improved, but one of the chicks developed a well-defined case of paralysis. In view of the unusually good performance of the negative control group, the appearance of paralysis on the 15 per cent mold ration probably means that this level is quite inadequate as a source of vitamin B_4.

The group getting ration 441A supplemented with 30 per cent mold made a rather slow start, but grew very rapidly during the final weeks of the experiment. None of the chicks in this group showed any signs of paralysis at any time during the 6 weeks period. As shown in table 1, this group attained an average weight of 419 gm. in 6 weeks. It seems quite safe to assume that 30 per cent of mold in the ration gives adequate protection against vitamin B_4 deficiency.

SUMMARY

The addition of 10 per cent of the mycelium of A. sydowi to a ration low in vitamin B (complex) furnished enough of the vitamin to support good growth in rats. Better growth and performance was secured on 20 per cent of the mold.

The mold contained enough vitamin $B(B_1)$ to protect chicks against polyneuritis when it was fed at a 10 per cent level.

Symptoms of pellagra were prevented in chicks on a ration low in vitamin G by the addition of 1 per cent of mold. To secure good growth of chicks it was necessary to add 3 per cent of mold.

Mold added at a level of 30 per cent to a ration deficient in vitamin B_4 completely prevented the onset of paralysis.

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THE NUTRITIVE VALUE OF FUNGI

III. THE GROWTH OF RATS ON SUPPLEMENTED AND UNSUPPLEMENTED MOLD PROTEINS [1]

H. J. GORCICA, W. H. PETERSON AND H. STEENBOCK
Departments of Agricultural Chemistry and Agricultural Bacteriology,
University of Wisconsin, Madison

FIVE FIGURES

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It has long been known that some of the filamentous fungi, especially the members of the genera, Aspergillus and Penicillium, are able to synthesize their cell proteins using inorganic salts as the sole source of nitrogen. This ability of converting inorganic nitrogen into protein nitrogen has led to speculations on the possibility of using mold proteins for food.

In Germany attempts were made to produce cattle fodder of increased protein content from straw by the use of a species of Aspergillus (Pringsheim and Lichtenstein, '20). In Japan the availability of mold proteins to albino rats was studied with the species which is used extensively in Japan in the manufacture of Koji-sauce, Aspergillus oryzae (Takata, '29). The protein of this mold was shown to be incomplete, and the addition of cystine did not correct the deficiency. Skinner ('34) recently reported that the proteins synthesized by Penicillium from an inorganic-glucose medium contained at least traces of the essential amino acids, but not in sufficient amounts to permit much growth in rats at a protein level of 9 per cent. At an 18 per cent level growth was improved, but still was not normal. When the ration containing 9 per

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[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

cent of mold protein was supplemented with 0.25 per cent cystine, growth was improved, but no figures were given as to the extent of the improvement.

In all problems in which the composition of molds is a factor, it is necessary to keep in mind the extreme variations with species and the variability in each species when grown on different media and under different conditions. Thus, in a survey of the composition of various molds carried out in this laboratory (Pruess, Eichinger and Peterson, '34), it was found that under identical conditions the crude protein (N × 6.25) content of a number of molds varied from 13.7 per cent to 43.7 per cent. Also, when grown on a glucose-inorganic salts medium, P. chrysogenum contained 43.7 per cent crude protein, while on a glucose-malt sprouts medium it contained only 15 per cent. A. oryzae which contained 33.7 per cent crude protein when grown on glucose-inorganic salts, increased this figure on glucose-malt sprouts to 36.3 per cent, although the average protein content of twenty-four molds was 30 per cent lower on the malt-sprouts medium than on the inorganic salts medium. The amount of lipid, sterol and carbohydrate in these molds also showed great variations.

There is, moreover, considerable evidence that even pure strains of molds will change their metabolism with long-continued propagation on stock agar media. A classical example of this phenomenon was the discovery by Wehmer (cited in Bernhauer, '32) that an Aspergillus which originally had produced fumaric acid subsequently degenerated, and completely lost the ability to form this acid.

In the light of the above considerations, there need be no contradiction between the findings of Takata ('29) and Skinner ('34), one of whom found that cystine did not remedy the deficiency of mold protein, while the other reported that cystine was the primary limiting amino-acid. The mere fact that the two investigators used different species of mold might well explain the discrepancy.

In the course of experiments previously carried out in this laboratory (Skinner, Peterson and Steenbock, '33), it was found that the proteins of four different molds were incapable of promoting growth of rats when fed at the 18 per cent level. When mold replaced part of the starch in a complete ration, the growth rate was reduced, but when mold was added to a ration containing only 4 per cent casein, growth was improved. The addition of small quantities of casein or yeast to rations containing 18 per cent mold protein greatly improved growth.

In making further studies of mold protein for the purpose of ascertaining the nature of the deficiencies, it was decided to concentrate attention on a single mold. The mold chosen was Aspergillus sydowi, a species which grows vigorously on a liquid synthetic medium.

EXPERIMENTAL

Preparation of mold mycelium. The mycelium used in these experiments was from the same strain of Aspergillus sydowi as that used in previous work in this laboratory (Skinner, Peterson and Steenbock, '33). It was grown on a glucose-inorganic salts medium of the following composition: 10 gm. NH_4NO_3, 6.8 gm. KH_2PO_4, 5 gm. $MgSO_4.7H_2O$, 0.16 gm. $FeCl_3.6H_2O$, 0.05 gm. $ZnSO_4.7H_2O$, and 100 gm. glucose made up to 1 liter with tap water. This medium was acidified with 1 cc. of 85 per cent H_3PO_4 per liter, placed in pans (10 liters per pan) in sterilizer-incubators (Peterson, Pruess, Gorcica and Greene, '33), and steamed at 100° for 8 hours. After the steam was turned off, a stream of sterile air was forced into each fermenter.

When the fermenters had cooled to room temperature, they were inoculated through the side openings with 100 cc. of a spore suspension of A. sydowi for each pan of medium. The temperature of the room was maintained at 30° for the incubation period, 10 days.

At the time of harvest the mold pads were rinsed once with cold water, pressed out by hand, and then stored in cans in a refrigerator at 5°F. Later, they were removed from the cans with a steel chopper, ground immediately to a fine paste and mixed with the other ingredients of the ration as needed. The rations were kept in tightly-covered containers in an electric refrigerator, from which they were removed only at feeding time.

Composition of rations. The basal ration had the following composition: 50 parts mold (dry basis), 44 parts cooked cornstarch, 4 parts mineral salts mixture no. 40 (Steenbock and Nelson, '23), and 2 parts cod liver oil. Mold was the only source of nitrogen in this ration. When protein supplements were added to the basal ration, a corresponding amount of starch was taken out. From previous experiments it was known that 20 per cent mold in the ration supplied ample amounts of the vitamin B complex.

Control of consumption. The animals used were young vigorous male rats of 50 to 60 gm. weight. Six rats were started in each group. Each animal was placed in an individual cage with a screen bottom. Each morning the unconsumed ration was weighed and discarded and a fresh portion was weighed out.

The intake of all the animals which were given a 5 per cent protein supplement was held for the first 4 weeks to that of the control group, for the remaining 6 weeks to that of the group getting 5 per cent skim-milk protein. The control group had the lowest intake throughout the experiment, but the rapid decrease in consumption by this group in the fifth and sixth weeks, together with the death of animals in the group, made it inadvisable to hold down the intake of the other groups to this level. Accordingly, for the last 6 weeks the intake of the others was controlled by that of the next limiting group, which was the group getting 5 per cent skim-milk protein.

Within the limiting groups the intake of the animals was held, in general, to that of the rat that ate the least. When the intake of one animal in the group fell below that of the preceding week, the other animals were not restricted to this level, but were held to that of the preceding week. The consumption of ration per animal per week by those getting 5 per cent protein supplements was as follows: 37, 32, 48, 44, 48, 56, 60, 64, 68, 70 gm., total, 527 gm.

When the groups getting 2½ per cent protein supplements were started, it was planned to limit the intake of each animal to 527 gm. over a 10-week period while keeping the weekly intakes of all animals equal. This plan was abandoned at the end of the seventh week when the intakes of all the animals began to decline, and during the last 3 weeks consumption was equalized only within groups. The weekly intake per animal was as follows: 40, 40, 42, 47, 50, 51, 56, 59, 65, 70 gm., total 520 gm. In cases where the intake varied from that given above, it will be referred to in the discussion.

Growth on protein supplements. For control purposes one group was given the basal ration unsupplemented, so that the mold in the ration was the sole source of nitrogen. The average growth curve of the group is shown in figure 1. All of the animals on the control ration died in from 4 to 7 weeks, but they all gained in weight before death—the average gain in weight of the group at time of death was 16 gm. Since the mold in the ration was known to supply ample quantities of the vitamin B complex (preceding paper), it is evident that the proteins of A. sydowi did not support life when the mold was fed at a level of 50 per cent, equivalent to 18 per cent of crude protein (N × 6.25).

The materials used as protein supplements were casein, dried skim-milk powder, whole wheat, corn gluten feed, whole corn, dry powdered egg white, soy bean meal, gelatin and yeast.

Casein was given as a protein supplement to three groups of rats at 10, 5 and 2½ per cent levels (fig. 1). Although the rats were limited to the same food intake as the control group, the average gain in weight of the first group was 131 gm., of the second group 101 gm., and of the third group 64 gm. The addition of as little as 2½ per cent casein markedly improved the ration. The intake, average gain in weight, and the gain in weight per gram of protein consumed are given in table 1.

Since cystine is the limiting amino-acid in casein (Osborne and Mendel, '15), it appears improbable that the additional cystine supplied by the casein at a 2½ per cent level prevented the early death of the animals and supported the greatly increased growth noted. It seems more likely that the factor responsible for this pronounced effect at such a low level must be some rather abundant constituent of the casein.

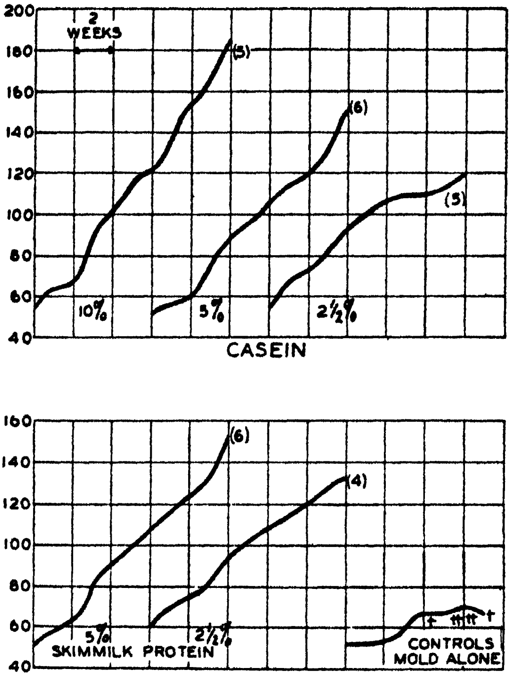

Fig. 1 Average growth curves of rats on mold as the sole source of protein and with supplements of casein and skim milk protein at various levels. The figures in parentheses indicate the number of individuals represented by the curve. The crosses beneath the curve representing the control group indicate the death of animals in the group.

Dry skim-milk powder was fed at levels such that the rations contained 5 and 2½ per cent skim-milk protein. The average gain in weight (fig. 1) was almost identical with that on the corresponding levels of casein. This fact provides further evidence that the mold is probably not deficient in cystine, since if cystine were the factor supplied by the supplements,

TABLE 1

Growth of rats on mold mycelium supplemented by certain proteins

TYPE OF PROTEIN ADDED	CASEIN			DRIED SKIM-MILK		CORN GLUTEN FEED		WHOLE WHEAT		EGG WHITE	
Level of supplement, per cent	2½	5	10	2½	5	2½	5	2½	5	2½	5
Ration consumed, grams	504	537	537	508	537	520	537	520	537	501	463
Total protein consumed, grams	103	124	150	104	124	107	124	107	124	103	106
Supplementary protein consumed, grams	12.6	26.9	53.7	12.7	26.9	13.0	26.9	13.0	26.9	12.5	23.2
Number of survivors after 10 weeks[1]	5	6	5	4	6	6	6	5	4	5	5
Average gain in weight, grams	64	101	131	72	99	111	101	100	118	66	73
Gain in weight per gram of supplementary protein consumed, grams	5.1	3.8	2.5	5.7	3.7	8.5	3.8	7.7	4.4	5.3	3.2
Gain in weight per gram of total protein consumed, grams	0.62	0.81	0.87	0.69	0.80	1.04	0.81	0.94	0.95	0.64	0.69

[1] Number of original animals (six).

the proteins of skim milk, due to their content of lactalbumin, should be considerably more effective than casein in promoting growth when added to mold.

The proteins of whole wheat were fed as a supplement at levels of 5, 2½ and ½ per cent. The average gains on the 5 per cent level were 118 gm. and on the 2½ per cent level 100 gm.

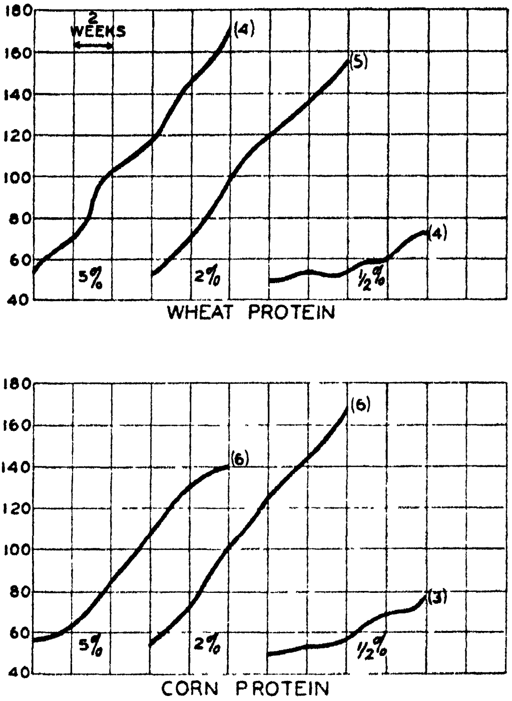

Fig. 2 Average growth curves of rats on mold supplemented by wheat and corn proteins at various levels. The figures in parentheses indicate the number of individuals represented by the curve.

(fig. 2). The animals getting ½ per cent wheat protein grew very slowly. At the end of 8 weeks the four survivors had gained on the average 23 gm. At this time two of the animals were given supplements of amino-acids. These animals will be discussed later.

Corn protein in the form of corn gluten feed (from Pennick & Ford, Cedar Rapids, Iowa) was fed at levels of 5 and 2½ per cent. Both groups grew well; the group on the lower level made an average gain of 111 gm., which is the best record made by any of the groups getting a 2½ per cent protein supplement. As shown in table 1, this group gained 1.04 gm. per gram of crude protein consumed. Inasmuch as the mold nitrogen is not more than two-thirds protein nitrogen (Gorcica, Peterson and Steenbock, '34), the protein of the ration was quite efficiently utilized.

One group was fed the proteins of whole corn at a level of ½ per cent, and in 8 weeks the three survivors had gained an average of 29 gm. The growth curves of this group are shown in figure 4.

Since Mitchell and Smuts have shown ('32) that the proteins of corn are deficient in lysine and tryptophane, and since the addition of only 2½ per cent of corn protein to the mold ration promotes good growth, the mold proteins are apparently not markedly deficient in either lysine or tryptophane.

Dried powdered egg white (Mallinckrodt's "Egg Albumin-Soluble-Impalpable powder") was fed at levels of 5 and 2½ per cent. The average total intake of the group getting 5 per cent egg white was only 463 gm., while the average gain of the group was only 73 gm. This was the smallest gain of any group getting a 5 per cent supplement of protein (except those getting gelatin), but it was undoubtedly due to the low intake. At the 2½ per cent level the intake was 501 gm. and the average gain 66 gm.

The growth on the ration supplemented with egg white was so much poorer than had been expected that it was decided to determine whether the egg white used was a raw or denatured preparation. The following ration was used: cooked cornstarch, 70; egg white, 18; mineral salts mixture no. 40, 4; agar, 2; cod liver oil, 2. Three rats were fed this ration made up with the same egg white as before, while three others were given this egg white after it had been cooked in an autoclave at 15 pounds pressure for 20 minutes. The growth curves of these animals are given in figure 3.

The animals getting raw egg white each consumed 400 gm. of ration in 10 weeks. Their average gain during that time was 23 gm. The animals began to lose hair after having been on the ration 5 weeks, and at the end of 10 weeks they had lost the hair over almost the entire body. Some of these symptoms were the same as those described by Parsons and Kelly ('33) in their paper on the toxicity of egg white to rats. The group getting autoclaved egg white made an average gain of 140 gm. in 10 weeks on an intake of 633 gm. These animals lost no hair and were in excellent health throughout the experiment. On the evidence of this experiment, it seems very likely that the preparation used was raw egg white, which fact explains the poor results obtained with it as a supplement to the mold.

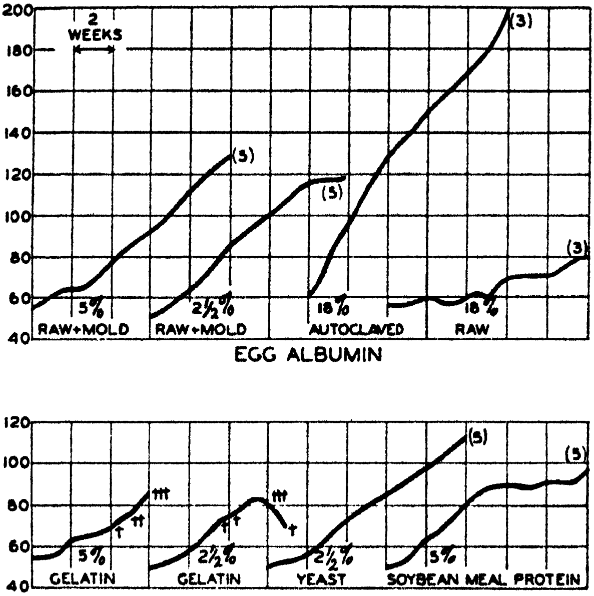

Fig. 3 Average growth of rats on mold supplemented with various proteins. Also the growth of rats on heated and unheated egg white as sole source of protein. Crosses indicate death of animals in the group.

The supplemental value of soybean protein was also determined. One group was given soybean meal protein at a 5 per cent level. The meal was a preparation that had been heated at 140° for ½ hour, and was known to be palatable to rats. The group made an average gain of 48 gm. in 10 weeks on an intake of 459 gm. (fig. 3). The intake of the group was declining rapidly during the last 2 weeks of the experiment.

Gelatin proved to be a very poor supplement. It was fed at two levels. When added at a 5 per cent level, the animals died in from 4 to 6 weeks, after making some growth which totalled 21 gm. at the time of death. When the ration contained 2½ per cent gelatin, five animals died in 3 to 6 weeks, while one lived for 9 weeks. The average gain in weight at the time of death was 27 gm. The growth curves of these two groups are shown in figure 3.

One group of rats was fed a supplement of yeast at a 5 per cent level, equivalent to 2½ per cent protein. The average gain of the group was 68 gm. on an intake of 537 gm. The average growth curve of this group is shown in figure 3. Yeast protein appears to have about the same supplemental value as casein or skim-milk powder.

Growth on amino-acid supplements. From the data given above it appeared that the mold protein was not deficient in lysine or tryptophane, nor markedly deficient in cystine, but suggested the possibility of a histidine deficiency. It was decided to try the addition of various amino-acids.

A group of rats was available that had been on a ration containing 18 per cent mold protein plus ½ per cent whole wheat protein or ½ per cent whole corn protein for 8 weeks. Of the surviving animals which had received the wheat supplements, two were continued on this ration as controls, and the other two were placed on a ration in which the wheat had been replaced by ½ per cent cystine plus 1 per cent tyrosine. The food intakes of the four rats were kept equal. The experiment was continued for 4 weeks. Of three rats which had received corn protein, one was continued as a control, while the other two were given ½ per cent cystine and 1 per cent tyrosine instead of the corn. The addition of cystine and tyrosine failed to improve growth (fig. 4).

A group of six rats, 85 to 103 gm. in weight, was started on a ration containing 18 per cent mold protein. After a rapid initial loss in weight, the animals began to grow very slowly, but at the end of 8 weeks their average weight was only 7 gm. more than when they were put on the experiment. Two of these animals were given a supplement of ½ per cent cystine, two others were given 1 per cent tyrosine, and two were given ½ per cent cystine plus 1 per cent tyrosine. In no case did the addition of these amino-acids cause any decided improvement in growth. It therefore seems quite definitely established that the addition of cystine and tyrosine to our mold did not improve its growth-promoting power.

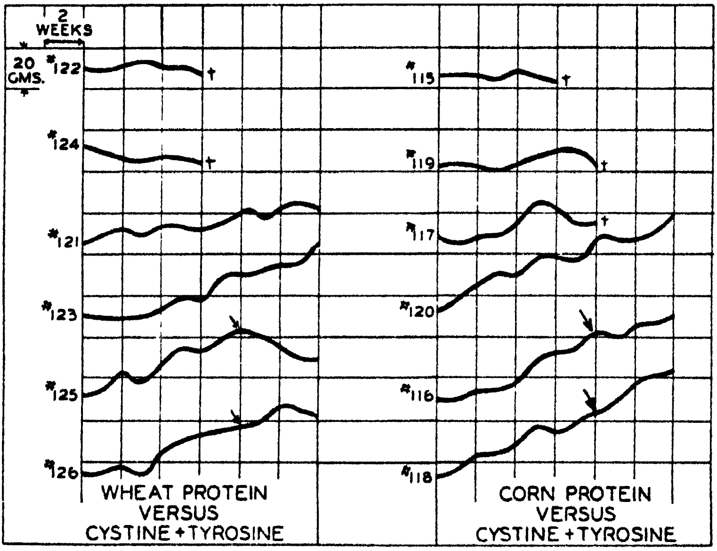

Fig. 4 Comparison of wheat and corn protein with cystine and tyrosine as supplements to mold protein. Arrows indicate point at which 0.5 per cent corn or wheat proteins were replaced by 0.5 per cent cystine plus 1 per cent tyrosine.

To test the effect of supplementing mold protein with histidine, a group of seven rats was started of which three as controls received 18 per cent mold protein, while the others were given in addition ½ per cent histidine. As shown in figure 5, three of the rats getting histidine grew more rapidly than the three control rats, but died in 6 weeks or less, while no. 153, which grew at about the same rate as the control animals, lived the longest. At the end of 7 weeks, it was obvious that the addition of histidine to the mold ration would not support continued growth and the group was therefore discarded.

It is evident, that other combinations of amino-acids or supplements must be tried before the deficiencies of the mold mycelium can be determined.

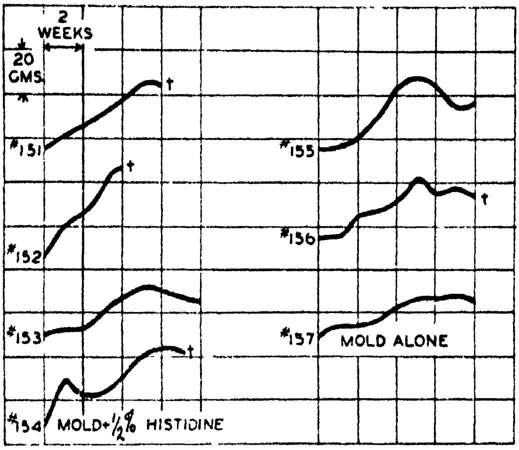

Fig. 5 Supplementary value of histidine to mold protein.

SUMMARY

The nutritive value of the proteins of Aspergillus sydowi, when produced on a synthetic medium (inorganic salts-glucose), was determined. The mold mycelium was fed as the sole source of protein or supplemented with various amounts of other proteins or with certain amino-acids.

The proteins of A. sydowi were found insufficient to promote more than a slight amount of growth or to sustain life in young rats for a period longer than 7 to 9 weeks.

The most effective supplements were found to be the proteins of whole wheat and corn gluten feed. When these were added to the basal ration at either a 2½ or a 5 per cent level, the resulting ration supported very good growth.

Supplementing the mold protein with casein, skim-milk protein, egg white and yeast protein at levels of 2½ and 5 per cent resulted in good growth, but growth was better at the higher level.

Supplementation with gelatin at 2½ or 5 per cent levels did not improve the growth-promoting power of the mold protein.

The addition of ½ per cent cystine, ½ per cent histidine, 1 per cent tyrosine, or ½ per cent cystine plus 1 per cent tyrosine, to a ration containing 18 per cent mold protein did not result in any improvement in growth.

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THE NUTRITIVE VALUE OF LACTOSE IN MAN

ALFRED E. KOEHLER, IONE RAPP AND ELSIE HILL
Santa Barbara Cottage Hospital and The Sansum Clinic, Santa Barbara, California

ONE FIGURE

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Information on the nutritive value of lactose in man is very meager and only indirect evidence is available. Berglund and Folin ('22) showed in one case that after giving 100 gm. of lactose orally to a subject there was only a slight or insignificant rise in the blood sugar. This inability of lactose to produce a hyperglycemia could be explained either by a failure of, or delay in, its absorption from the alimentary tract or by its rapid combustion or storage. Higgins ('16) studied the effects of the ingestion of lactose on the respiratory quotient and oxygen consumption and found the respiratory quotient well above 1.00; in fact, even considerably higher than after the administration of an equivalent amount of glucose. Benedict and Carpenter ('18) also obtained in man a specific dynamic effect following lactose administration equal to or greater than that of glucose. More recently, McCullagh and McCullagh ('32) showed that lactose had an effect similar to glucose in lowering inorganic blood phosphorus and in decreasing its excretion in the urine. The exact significance of the inorganic blood phosphate changes after sugar ingestion is unknown, but the lowering of the phosphate curve has usually been looked upon as evidence of sugar utilization. The above investigators found, however, that galactose does not alter the blood inorganic phosphate or its urinary excretion, although it can be utilized by the body at least to a considerable extent. From the standpoint of absorption of lactose from the alimentary tract the early observations of Bourguelot and Troisier (1889) are interesting. They fed lactose to a diabetic subject and recovered in the urine two molecules of glucose for each of lactose ingested and no galactose was detected.

It can readily be seen that the information on lactose utilization from the nutrient standpoint in man is altogether inadequate. The data available is based on indirect evidence, the physiological significance of which is still uncertain.

PLAN OF STUDY AND METHODS

The glycemic response after lactose ingestion was studied in normal, obese, and diabetic subjects. The value of this was emphasized after a few subjects were studied, inasmuch as there was not only considerable variation in apparently normal cases, but a greater variation was noted when diabetic and obese subjects were compared with the normal group. Lactose was given to subjects (1.5 gm. per kilogram) in the morning in a fasting state. Venous blood and urine samples were collected hourly for 4 hours. Except in a few cases, the subjects were at rest, either lying or sitting during the period of study. If the urine specimen gave a positive reduction test, the fermentability of the sugar was determined. The Hartman-Shaffer method for blood sugar was used.

In a group of normal cases the blood inorganic phosphate changes after lactose ingestion were also studied. This work was started before the observations of McCullagh and McCullagh ('32) came to our attention and then it was decided to extend their observations on a few cases to a larger series.

RESULTS

Glycemic effect. As a basis for comparison, particularly from the standpoint of individual variation and average values, the blood sugar and urinary excretion after glucose ingestion was studied in twenty-six apparently normal cases. The average glycemic response is shown in figure 1. Considerable variation was noted, but the average values form a reliable basis for comparison. The comparable results after lactose ingestion in eighteen normal young adults are shown also. Although 80 to 120 gm. of lactose were given in a single dose, only in one case was there flatulency and diarrhea. The individual variations in the blood sugar values were again quite large, comparable to those obtained with glucose. In general, lactose ingestion resulted in no appreciable increase in blood sugar and the average values show quite clearly the lack of any hyperglycemic effect. The average values for the first, second, third and fourth hours after lactose are sur-

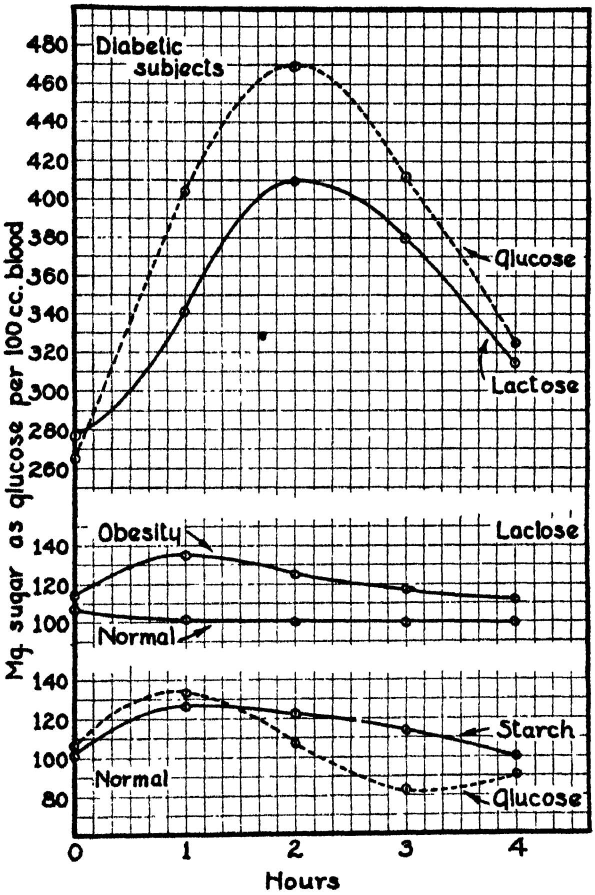

Figure 1

prisingly constant. The secondary hypoglycemic effect usually found at the time of the third hour after glucose ingestion, was much less, practically insignificant, after lactose. Glycosuria was much more frequent after lactose than after glucose administration, but the excreted sugar was nonfermentable in all cases studied and apparently was lactose that was assimilated as such from the intestinal tract, although the possibility of this having been galactose was not eliminated.

The average glycemic response in six normal adults after the ingestion of a comparable amount of starch is shown to compare the rate of sugar absorption after the hydrolysis of a polysaccharide. At the end of the first hour the blood sugar rise after starch was only a little less than that after glucose ingestion, but the hyperglycemia was more prolonged for starch and the secondary hypoglycemia was absent as in the case of lactose. Our results are consistent with those of Kjer ('24), who found that starch caused quite as great a hyperglycemia as did an equivalent amount of glucose.

The results of lactose tolerance tests in a group of obese subjects are also shown. The hourly averages after lactose ingestion in sixteen overweight cases showed a definite rise for the first and second hours. This rise was, however, not universal for all obese cases, for in some there was even a lowering of the blood sugar after lactose administration. In some obese cases the rise in blood sugar was of the magnitude found in mild diabetics after glucose ingestion. The effect of fasting and mechanical stimulation of peristalsis was studied in two subjects after the ingestion of 1.5 gm. powdered cellulose per kilogram body weight. In neither case was there any appreciable change in blood sugar. The rise of the blood sugar in the majority of obese cases indicated that in all probability there is at least partial assimilation of ingested lactose.

The effect of lactose ingestion in eight cases of moderately severe diabetes mellitus is also shown in figure 1. The marked increase in the blood sugar in the first, second, third and even fourth hours after lactose administration is apparent. In all these cases the blood sugar increase over the fasting specimens was accounted for as fermentable sugar. In these subjects the equivalent amount of glucose was also administered and no very marked differences were obtained between the blood sugar curves after lactose or glucose. The peaks for the lactose and glucose curves were at approximately the same time indicating that there was no gross delay due to splitting of the lactose. In one case a control glycemia curve was obtained after the equivalent amount of powdered cellulose ingestion and little variation in the 4 hours after fasting was noted. It was of particular interest to note that, in spite of considerable excretion of 'extra sugar' in the urine, as much as 13.38 gm. for the third hour in one case, the urinary sugar in every instance was all fermentable. In other words, lactose or galactose was not excreted in the diabetic as it usually was in the normal individual after lactose ingestion. Bourquelot and Troisier had already pointed this out in one case in 1889, although their analytical methods were unrefined as compared with those of the present day. They fed lactose to a diabetic patient and recovered two molecules of glucose in the urine for each one of lactose fed. It would seem from these experiments that the diabetic not only assimilates lactose, but that he also completely converts, at least the excreted sugar, to glucose. This latter phenomenon is, to our knowledge, the only specific example of the ability of the diabetic to convert a substance to glucose which normally may be excreted in another form (lactose or galactose). This observation is of interest from the standpoint of the overproduction hypothesis as a causative factor in diabetes.

The recovery in the urine of lactose administered intravenously. The failure of the diabetic to excrete lactose raised the question of his ability to convert intravenously injected lactose. The lactosuria in the normal after lactose ingestion is undoubtedly due to the absorption of lactose as such from the bowel. There is no reason to believe that such an absorption should not also take place in the diabetic and its failure of being excreted points to its conversion or destruction.

Table 1 gives the amount of lactose recovered in the urine as non-fermentable sugar after a known amount of lactose was slowly injected as a 10 per cent solution. In five normal cases the average per cent recovered was 93.2, while in three obese cases 89.7 per cent was recovered. The small decrease in the recovery in the obese group is probably insignificant because of the greater dilution of the same amount of lactose in the blood and tissue fluids in these subjects as compared with normal individuals. In two diabetic subjects studied the recovery of excreted lactose was approximately the same as in the normal or obese groups. The injection of 30 units of insulin 45 minutes before the lactose injection in the case of M.F. had no effect on the lactose recovery. Apparently the diabetic subject has no greater power to destroy intravenously administered lactose than the normal individual. Consequently other factors must play a role in the absence of

TABLE 1

The recovery in the urine of lactose administered intravenously

CASE	AMOUNT GIVEN	AMOUNT RECOVERED	PER CENT RECOVERED
		Normal	
	gm.	*gm.*	
A. E. K.	15.5	14.1	91
G. B.	14.2	14.0	98.5
G. W.	14.2	12.3	86.5
D. Mc.	14.0	13.8	98.7
B. W.	13.1	12.0	91.5
Average	14.2	13.2	93.2
		Obese	
A. W.	14.0	12.3	87.9
E. B.	15.0	14.0	93.4
E. W.	14.6	13.0	88.0
Average	14.4	13.1	89.7
		Diabetic	
M. F.	14.6	13.0	88.0 (with insulin)
M. F.	15.5	13.7	88.5 (without insulin)
G. W.	13.1	12.0	91.5
Average	14.3	12.9	89.3

lactosuria in the diabetic after lactose ingestion and a difference in the process of alimentation may be suspected.

The possibility of liver lactase in the human as has been found by Cajori ('34) in the liver of the dog must be kept in mind. This may account for 10 per cent of the lactose that was destroyed after intravenous administration.

The effect of lactose on the inorganic phosphate content of the blood. The effect of glucose administration on the inorganic phosphate content of the blood is well known. Our results shown in table 2 substantiate the findings of McCullagh and McCullagh ('32) that lactose lowers the blood inorganic phosphate, although in our series of eight normal subjects the average decrease was only 0.4 mg. per 100 cc. of blood. This small change must not necessarily be interpreted as indicating lactose utilization.

TABLE 2

The effect of 1.5 gm. lactose per kilogram orally on the inorganic phosphate content of whole blood. (Expressed as milligrams per 100 cc. of blood)

CASES	FASTING	FIRST HOUR	SECOND HOUR	THIRD HOUR	FOURTH HOUR
E. H.	4.1	3.3	2.9	2.9	3.1
F. H.	3.3	3.3	3.2	3.3	3.3
C. H.	2.9	2.9	2.6	2.9	3.3
M. Mc.	2.6	2.5	2.5	2.8	3.0
K. O.	2.9	3.0	2.9	3.0	3.0
M. H.	3.7	3.6	3.7	3.6	3.6
I. C.	3.6	2.9	3.0	3.1	3.3
E. P.	3.0	2.2	2.4	2.4	2.7
Average (8 cases)	3.3	3.0	2.9	3.0	3.2

DISCUSSION

The average blood sugar values in normal individuals after lactose ingestion showed quite clearly the absence of any hyperglycemic effect. Such a failure to elevate the blood sugar might well be due to increased rate of utilization and storage or to delayed absorption and consequently offers no proof as to whether or not lactose is absorbed from the alimentary tract. If the failure of hyperglycemia is due to delayed absorption from slow hydrolysis, then the rate of splitting of lactose must be very much slower than that of starch, for the latter causes a rise in blood sugar as quickly and nearly as great as obtained after glucose ingestion. Such slow splitting of lactose together with the rapid rate that it may pass through the small intestine (Hosoi, Alvarez, and Mann, '28) may result in a portion or even a greater part of ingested lactose reaching the colon for bacterial utilization. That this probably is the case in the adult rat was shown by Koehler and Allen ('34) in experiments showing that lactose had a nutritive value inferior to that of glucose or sucrose on isocaloric diets.

In the diabetic, and to a lesser extent in the obese individual, however, there can be no doubt as to the marked absorption of lactose. At first it seemed possible that through some unknown mechanism lactose might give rise to a hyperglycemia due to mechanical stimulation of the intestines. This possibility was ruled out, however, by the feeding of powdered cellulose. It is difficult to conceive that the enzyme lactase would be present in the diabetic and not in the normal individual. The complete absence of lactosuria or galactosuria in the diabetic after lactose ingestion as compared with its usual presence in the normal individual suggests a difference in the alimentary assimilation, particularly since the diabetic handles intravenously injected lactose much the same as the normal individual.

In considering the possibility of the failure of lactose absorption, it should be remembered that besides the question of the hydrolysis of this disaccharide, the effect of the intestinal rate on the absorption of foodstuffs should be considered. Hosoi, Alvarez and Mann ('28) have shown that lactose passes through the small intestine with great rapidity. Such a rapid rate could easily interfere with hydrolysis and absorption even though lactase were present. Not only could the increased intestinal rate caused by lactose delay its absorption, but it could also interfere with the absorption of other foodstuffs.

SUMMARY

1. The average blood sugar values in sixteen normal young adults after ingestion of 1.5 gm. lactose per kilogram body weight showed no important changes, although considerable individual variations were noted. The secondary hypoglycemic effects were lacking. There was frequently a lactosuria present.

2. The ingestion of 1.5 gm. starch per kilogram caused nearly as much of a peak rise in the blood sugar as obtained after the administration of glucose. The total hyperglycemia after starch is greater than after glucose.

3. The average blood sugar values in sixteen obese individuals showed a slight but definite elevation after lactose ingestion.

4. The blood sugar changes after lactose ingestion in the diabetic were marked and of nearly the same magnitude as after glucose ingestion. In the diabetic cases studied, lactosuria was never noted.

5. The average recovery in the urine of intravenously injected lactose was approximately the same (89.3 to 93.2 per cent) in normal, obese and diabetic subjects.

6. Ingested lactose caused a small fall in the inorganic phosphate content of whole blood.

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RELATION OF CASTRATION TO VITAMIN A-DEFICIENCY IN THE RAT[1]

K. E. MASON AND J. M. WOLFE

Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

TWO FIGURES

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It has been demonstrated that an adequate supply of vitamin A is essential for the maintenance and structural integrity of the germinal epithelium of the testis of the rat (Mason, '30, '33; Evans, '32). On the basis of these observations, it seemed of interest to determine whether the amount of A actually utilized by the testis was of sufficient magnitude to produce a significant delay in the appearance of the deficiency symptoms in rats completely castrated before being placed on vitamin A-deficient diets, as compared with non-castrate rats.

In a series of experiments involving seventy-eight rats and conducted during the fall and winter of 1929–1930, observations made incidental to other studies for which the animals were being prepared suggested that xerophthalmia was retarded in its appearance in castrated male rats, when compared with their non-castrate brothers (Wolfe and Mason, '30). The eye changes appeared in the castrates at about the peak of the growth curve; whereas in non-castrate rats these changes appeared some time before the growth peak was attained. However, similar observations were subsequently noted in a series of ten castrate female rats and ten non-castrate littermate sisters. Since it did not seem possible that

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[1] This investigation was aided by grants from the Commonwealth Fund and from the Division of Medical Sciences of the Rockefeller Foundation.

the ovary utilized sufficient vitamin A to produce any significant difference in the manifestation of the deficiency, it was thought that an altered metabolism following castration might afford a more logical explanation.

To test this hypothesis, the respiratory quotient and basal metabolic rate were determined on a series of rats including controls, castrated controls, A-deficient non-castrates and A-deficient castrates.[2] By careful regulation of the diet, a constant and moderately severe degree of xerophthalmia was maintained in the A-deficient rats during a period of from 60 to 100 days prior to the metabolic studies. The data obtained failed to demonstrate any metabolic differences sufficiently significant to explain the delayed appearance of xerophthalmia in castrated rats (table 1).

TABLE 1

Respiratory quotient and metabolic rate of A-deficient and control rats

RAT	TREATMENT	RESPIRATORY QUOTIENT		BASAL METABOLIC RATE		
		First determination	Second determination	First determination	Second determination	Average
41-15[1] ♂	-A	.74		772		839
-16 ♂	-A	.72		906		
41-17 ♂	-A castrate	.75		1095		989
-18 ♂	-A castrate	.73		883		
41-19 ♂	Control	.74		1001		1038
-20 ♂	Control	.74		1075		
41-21 ♂	Control castrate	.75		888		934
-22 ♂	Control castrate	.75		980		
40-50 ♂	-A castrate	.76	.76	898	1103	1001
-61 ♀	-A castrate	.77	.74	1076	975	1026
-68 ♀	-A castrate	.75	.75	831	930	881
-70 ♀	-A castrate	.73	.74	870	880	875

[1] Rats 41-15 to 41-22 were all litter-mate brothers.

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[2] Acknowledgment is made to Dr. L. G. Wesson, of the Department of Pharmacology, for his kindness and cooperation in making these metabolic studies. The method used was essentially that previously described by Wesson ('27).

In our earlier studies (Wolfe and Mason, '30) examination of the eyes was made bi-weekly, and not always by the same individual; which made it difficult to interpret the exact time at which xerophthalmia appeared. Furthermore, certain of the animals exhibited a mild chronic type of deficiency with temporary appearance of early signs of xerophthalmia at intervals, before more advanced stages became apparent. The inadequacies in the data warranted repetition of these experiments with more careful observations on the eye changes. During the following year, careful daily examination of the eyes of a series of sixteen A-deficient male rats, half of which were castrated before being placed on the experimental diet, gave no significant difference in the time of appearance of the eye changes in the two groups. It then seemed advisable to restudy the whole problem using female rats, in order to apply another criterion of A-depletion, namely, the abnormal vaginal cornification first described by Evans and Bishop ('22) and now generally accepted as a reliable indicator of vitamin A-deficiency. The results of these newer studies are outlined below.

The 102 rats selected for this study (series A and B, table 2) averaged approximately 40 gm. body weight (minimum 37 —maximum 46) and varied in age from 19 to 26 days when put on experiment. Both albino and pied strains were used, with no observable difference in the results secured. Litter-groups were equally divided between castrates and non-castrates, except where odd numbers were present. In the latter instance, the odd member was placed in the non-castrate group. Castration was performed just prior to the beginning of experimental feeding. The diets used were essentially the same as those used in earlier studies mentioned above. Their composition was as follows:

Diet 66

	Per cent
Casein (purified)	18
Starch	58
Lard	20
Salt mixture	4

Diet 67

	Per cent
Casein (unextracted)	18
Starch	58
Lard	20
Salt mixture	4

TABLE 2

Showing the average time of appearance of continuous vaginal cornification and xerophthalmia in A-deficient castrate and non-castrate rats

SERIES		NUMBER OF RATS	WHEN STARTED		PERIOD ON EXPERIMENT BEFORE CONTINUOUS VAGINAL CORNIFICATION			PERIOD ON EXPERIMENT BEFORE DEFINITE AND CONTINUOUS XEROPHTHALMIA APPEARED			INTERVAL
			Average weight, grams	Average age, days	Days	Minimum	Maximum	Days	Minimum	Maximum	
Series A (diet 65)	Non-castrates ♀	26	40.4	23.5	21.2	15	26	33.7 (22 rats)	25	44	10.2
	Castrates ♀	21	41.7	22.5	22.5	18	27	31.6 (20 rats)	25	42	9.1
	Non-castrates ♂	7	41.3	24.0	....	..	..	32.0	24	41	....
	Castrates ♂	6	42.3	24.0	....	..	..	32.0	26	40	....
Series B (diet 67)	Non-castrates ♀	24	41.5	21.0	21.7	17	27	43.0 (22 rats)	30	48	21.3
	Castrates ♀	18	43.1	21.0	23.1	17	27	42.5 (17 rats)	30	50	19.4
Series C (diet 67)	Non-castrates ♀	34	40.0 min. 37 max. 43	23.0	22.5	19	26	34.8	25	45	12.3
Series D (diet 67)	Non-castrates ♀	48	65.0 min. 50 max. 78	28.0	40.4	35	55	Used for other experiments after the seventieth day. At that time xerophthalmia had not appeared in any of the group			

In addition to the above diet, each rat received, by separate daily administration, 0.5 to 1 gm. of dried yeast and 0.005 gm. of irradiated ergosterol.

Daily vaginal smears (dried and stained in hematoxylin and eosin) were begun on the fourteenth day of experiment, the vaginal membrane being ruptured if not already open. The eyes were examined daily with the aid of a strong hand lens and good illumination. Body weights were recorded on alternate days. The rats were maintained throughout the experiment in individual, false bottom, wire cages in an airy well-lighted room, under uniform experimental conditions. All observations on the vaginal smears and eye-changes were made by the same individual.

From the results of series A (table 2), represented graphically in figure 1, it is apparent that continuous vaginal cornification not only appeared much earlier, but also showed much less variation in time of appearance than did the eye changes. Furthermore, the average time of appearance of both symptoms of A-deficiency was essentially the same for the castrate and non-castrate groups, the small differences being no greater than one might expect in determining the midpoint of the transition period between normal and abnormal vaginal or eye conditions. When careful comparisons were made between castrates and non-castrates from the same litter group, no significant differences in the time of onset of the deficiency symptoms were observed.

Similar results were obtained in the rats of series B, in which unextracted casein was used.[3] It is of interest to note that, although continuous vaginal cornification appeared at approximately the same time in both series, the small traces of A in the unextracted casein of series B significantly retarded the appearance of xerophthalmia and, in some instances, either no clear-cut eye symptoms appeared or else mild symptoms were observed to alternate, every few days, with complete absence of xerophthalmia. In all the rats of series A, and in the majority of those of series B, once the eye symptoms appeared they became progressively worse and reached their maximum severity within 2 weeks or less.

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[3] We have repeatedly demonstrated that the casein used in our studies during the past 6 years, a finely pulverized product obtained from A. Adler & Sons, Philadelphia, contained only small traces of vitamin A. Unless a very rapid and severe deficiency was desired in the experimental animals, the difficulty and expense of alcoholic extraction has not seemed warranted. In this connection, we would like to correct a statement made in a previous report (Mason, '33, p. 172, footnote 10), stating that the fat content of the above casein was 0.04 per cent. This was intended to refer to the extracted, and not the commercial, product.

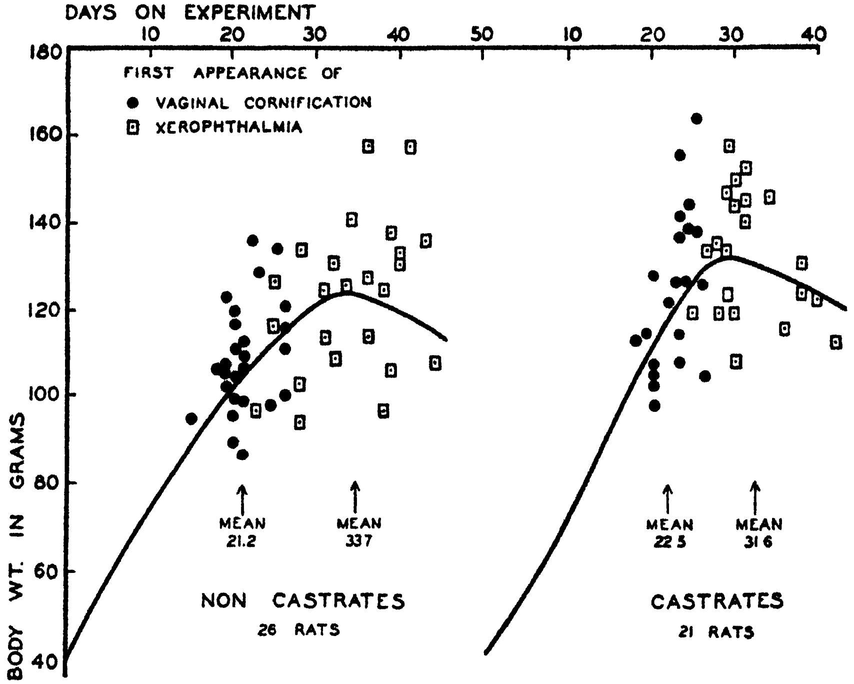

Fig. 1 Showing the average growth curve and the time of appearance of continuous vaginal cornification and of xerophthalmia in the individual A-deficient female rats of series A, table 2.

Only seven non-castrate and six castrate males were used (series A). Xerophthalmia appeared at approximately the same time in all instances. Since this was in accord with previous findings on a group of sixteen male rats, half of which were castrated, and confirmed similar observations on the female rats of series A and B, additional studies on males did not seem necessary. On the basis of the data presented above, it can be concluded that complete castration of the male or female rat had no demonstrable effect upon the development of vitamin A-deficiency.

The combined data from 126 rats, 114 of which are included in series A, B and C (table 2), presented graphically in figure 2, convincingly demonstrate the superiority of the vaginal smear test as a criterion of early vitamin A-deficiency. These rats weighed approximately 40 gm. when placed on the experimental diet. The average time required to induce continuous cornification was 22.2 days, with a maximum variation of 17 days; whereas the average time before xerophthalmia appeared was 37.3 days, with a maximum variation of 32 days. In other words, rats placed on a diet relatively low in vitamin A after attaining body weights of 40 gm. developed continuous vaginal cornification approximately 2 weeks prior to the appearance of definite xerophthalmia.

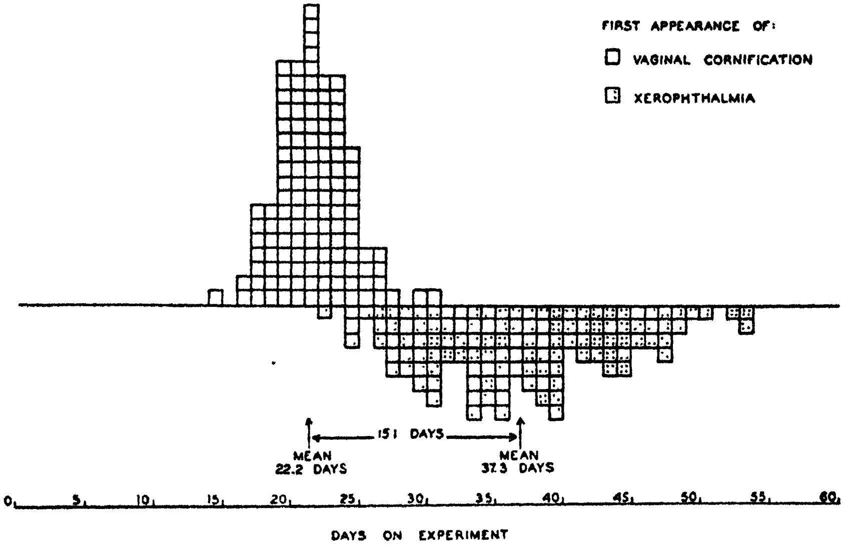

Fig. 2 Showing the distribution frequency of the time of appearance of continuous vaginal cornification and of xerophthalmia in 126 A-deficient rats (castrate and non-castrate) including 114 of those of series A, B and C (table 2).

In general, the earlier the onset of vaginal cornification and of xerophthalmia, the smaller the time interval between the appearance of these two criteria. We have frequently noted an almost simultaneous appearance of these criteria before the end of the suckling period in young rats whose mothers were maintained on low levels of vitamin A-intake. When rats from the stock colony, with a moderate storage of vitamin A and weighing approximately 40 gm., were placed on the experimental diets (series A, B and C, table 2) the average of the interval between the appearance of the two symptoms varied from 9.1 to 21.3 days. When young rats weighing from 50 to 75 gm. were used (series D, table 2) the interval became much greater. The rats of this latter series were used for other studies before xerophthalmia developed. From our experience with other groups, in which the experimental conditions were essentially the same, xerophthalmia would have appeared between the eightieth and ninetieth days. This gives an interval of approximately 45 days between the appearance of vaginal cornification and of xerophthalmia.

DISCUSSION

The studies presented in this paper amply confirm previous observations of Evans and Bishop ('22), Baumann and Steenbock ('32), Aberle ('33) and others in regard to the sensitivity and reliability of continuous vaginal cornification as a criterion of early vitamin A-deficiency. They further indicate that castrate rats offer no additional advantage over non-castrate rats, in ascertaining the onset of the abnormal changes. It should be stated, however, that in the present studies, involving comparison of castrate and non-castrate litter-mate sisters (series A and B, table 2), abnormal cornification appeared before the first oestrus in 78 per cent of the non-castrate rats and before the approach of the third oestrous period in the remainder. There is no evidence that a greater degree of oestral activity in the vaginal epithelium prior to the onset of A-deficiency changes would have altered the time of appearance of the latter, resulting in a greater difference between castrates and controls in this respect.

On the other hand, the castrate rat has proved much less satisfactory than the non-castrate in following the repair of the vaginal cornification after vitamin A-therapy, as will be discussed more fully in a later report dealing with the histological details of the vaginal smear after A-deficiency (Mason and Ellison, '35). The slightly superior growth of the castrates in the present series (fig. 1) as well as in the earlier studies mentioned above, was first thought to be due to the intentional selection of the larger animals of each litter group for castration with the idea of thereby offsetting a possible early retardation due to the operation. But the latter seemed to have no significant effect on the subsequent growth of the animals. This may be better explained by the recent observations of Beebe ('32) that "an increase in weight and in food consumption occurs in rats following ovariectomy, even in the face of vitamin A-deficient diets. The beneficial effect of ovariectomy is especially pronounced when performed early in life."

CONCLUSION

1. Complete castration caused no significant difference in the time of appearance of continuous vaginal cornification or of xerophthalmia in A-deficient rats of either sex.
2. Continuous vaginal cornification was subject to much less individual variation, and could be detected approximately 2 weeks earlier, than xerophthalmia, in rats placed on the diet at body weights of 40 gm.
3. Castrate rats were not superior to non-castrates for determining the beginning of vaginal cornification.

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CHANGES IN THE VAGINAL EPITHELIUM OF THE RAT AFTER VITAMIN A-DEFICIENCY[1]

K. E. MASON AND E. T. ELLISON
Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

TWO PLATES (TWENTY FIGURES)

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Excessive cornification of the vaginal epithelium in the rat is a characteristic and early manifestation of vitamin A-deficiency. The first observation of this phenomenon by Evans and Bishop ('22) has been repeatedly confirmed by many investigators. By the majority of these, the continued presence of cornified cells in the vaginal smear has been regarded as a reliable index of early depletion of vitamin A (Macy, Outhouse, Long and Graham, '27; Hohlweg and Dohrn, '30; Baumann and Steenbock, '32; Aberle, '33; Mason and Ellison, '34; Mason and Wolfe, '35). Others have considered this phenomenon unsatisfactory for diagnostic purposes (Coward, '29; Coward, Morgan and Dyer, '30; Thatcher and Sure, '32). This whole problem is more completely reviewed in a recent report by Baumann and Steenbock ('32), to which the reader is referred.

Normally, cornified cells appear in the vaginal smear of the rat only during about one-third of the total oestrous cycle (oestrus and metoestrus). Their constant presence in A-deficient rats does not indicate a continuous state of oestrus, for ovulation and corpora lutea formation occur at regular or irregular intervals, as indicated by successful matings

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with normal males (Evans and Bishop, '22; Evans, '28). Except for the latter studies, no attempt has been made to demonstrate rhythmic oestrous changes in A-deficient animals.

In an extensive analysis of the effect of vitamin A-deficiency on the female reproductive system of the rat, representing a continuation of similar studies on the male rat (Mason, '33), we have had occasion to study more than 52,000 vaginal smears obtained from more than 500 A-deficient and normal rats during the past 4 years. In preliminary studies (Mason and Wolfe, '35), it was demonstrated that abnormal cornification of the vaginal smears usually appears much earlier than do the other criteria of vitamin A-deficiency, and affords a very sensitive and reliable index of sub-xerophthalmic levels of the deficiency disease.

In the present report, the essential alterations observed in the vaginal smears during the progressive development of vitamin A-deficiency, and during repair of the latter, are described; and an interpretation of the manner in which lack of A influences the vaginal epithelium is offered.

METHOD

In making routine dry-stained vaginal smears on a large number of rats, the following method has proved most satisfactory. The numbers of the rats to be smeared are marked on glass slides, ruled transversely into twelve to fifteen rectangular areas, by means of a diamond pencil. The vaginal scrapings, obtained by means of a narrow aluminum spatula, are smeared as evenly as possible on the space opposite the proper number. Between each smearing, the spatula is thoroughly cleaned. Care should also be exercised in procuring the smears such that cornified cells from the external surface of the vaginal orifice are not included.

The smears are stained with Ehrlich's or Delafield's hematoxylin for ½ to several hours, washed gently with water, deeply blued with ammonia vapor, washed, faintly counterstained in eosin, washed and dried. It is important to secure an intensely blue stain sufficiently contrasted with, but not overshadowed by, the eosin counterstain. Microscopic examination of the entire smear is necessary, because of the variation in the proportion of different cell types in different parts of the smear. From this composite picture, the general characteristics of the smear can be estimated.

The diets used were composed of casein, starch, lard or Crisco, salt mixture, dried brewer's yeast, and irradiated ergosterol, and were of essentially the same composition as those used in earlier studies (Mason, '33; Mason and Wolfe, '35). Vitamin E was supplied by separate administration of wheat germ oil with the yeast supplement. When introduction of traces of vitamin A was not desired, the wheat germ oil was aerated at 100°C. for a period of 4 hours.

A. ABNORMAL VAGINAL CORNIFICATION OF A-DEFICIENCY

When the rat is rapidly depleted of vitamin A the interval between the first appearance of abnormal vaginal smears and the onset of complete and continuous vaginal cornification varies from a few days to a week. The description of the successive stages of cornification, as outlined in this paper, is based upon the vaginal smears of rats in which vitamin A-deficiency developed less acutely, in order to bring out more clearly the progressive changes which occur.

Early cornification. The first appearance of abnormal cornification in the dioestrous type of smear (fig. 1) was indicated by variable degrees of swelling, vacuolation and keratinization of the nucleated epithelial cells (fig. 2), and by a thin watery appearance of the mucus when present in such smears (fig. 3). Usually cornification was complete in only a relatively small number of the cells. Since such smears frequently resembled those of late metoestrus (fig. 8), continued cornification of the smear during several successive days was necessary for the differentiation of these two conditions. These early changes, and the increasing degrees of cornification which follow, were most clearly portrayed during prolonged periods of dioestrus; such as occur after castration and during pregnancy, pseudopregnancy and lactation. When the cyclic changes of oestrus occurred, the progressive cornification of the smears was interrupted by the normal processes of epithelial cornification, dehiscence and regeneration resulting from hormonal influences. The modification of the latter during the early stages of A-deficiency cornification will now be considered.

The normal oestrous cycle of the rat usually varies from 4 to 6 days in length, the general average approximating 5 days (Long and Evans, '22). Successive daily smears usually show the stages of prooestrus (figs. 5 and 6), oestrus (fig. 7), and metoestrus (fig. 8), followed by several days during which the smear of dioestrus (fig. 1) appears. Only infrequently does the smear of oestrus appear on 2 successive days.

As the vaginal epithelium became altered by lack of vitamin A, striking changes occurred in the prooestrous smear. Frequently, these changes were detectable at the transition period between late dioestrus and early prooestrus, in the pale swollen appearance of many of the large nucleated epithelial cells and in the watery appearance of the mucus, when present (fig. 4). At other times, the large basophilic nucleated epithelial cells so characteristic of normal prooestrus (fig. 5) appeared greatly swollen, and exhibited considerable cornification. The nuclei were very pycnotic and the cell outlines were usually rather fuzzy and indistinct (fig. 9). This type of smear somewhat resembled that of the transition period between normal prooestrus and normal oestrus (fig. 6), but was distinguishable from the latter by the larger size and more cornified nature of the epithelial cells. As the deficiency progressed, the atypical prooestrous smear soon became difficult to distinguish from that of oestrus. Its early obliteration afforded a striking and trustworthy criterion of early cornification of A-deficiency, and was usually followed by an apparent prolongation of oestrus over 2 successive days.

Moderate cornification. Following the appearance of the smears of atypical prooestrus, and of prolonged oestrus, the smears of dioestrus became more and more indistinguishable from those of metoestrus. They were characterized by an increase in the number of completely cornified nucleated epithelial cells and, particularly, by the predominance of large and small nucleated epithelial cells exhibiting intermediate stages of cornification (fig. 10). The acidophilic staining reaction of the majority of the epithelial cells gave a somewhat pinkish tinge to the entire smear. Not more than 10 to 15 per cent of the nucleated epithelial cells remained unaltered. Leucocytes were variable in number, representing from 20 to 40 per cent of the cells present. Mucus never appeared in the smear after early cornification became manifest. The material removed each day, by gentle insertion of the spatula, was cheesy in consistency and became more and more abundant as the cornification progressed.

Advanced cornification. As the cornified smear of pro-oestrus merged with those resembling prolonged oestrus, the oestrous cycle was represented by approximately three successive daily smears in which cornification was complete, or practically so, alternating with approximately two successive daily smears in which a relatively small number of leucocytes and partially cornified cells made their appearance among the completely cornified cells. The latter represented the increasing cornification of the dioestrous type of smear (figs. 11 and 12), in which the marked reduction in non-cornified nucleated epithelial cells was much more characteristic than was the relative number of completely cornified cells and leucocytes present.

Extreme cornification. As the deficiency progressed, the smear of dioestrus contained a still greater abundance of large flaky cornified cells but very few leucocytes and partially cornified cells (fig. 13). Eventually, the dioestrous smear contained only cornified cells (fig. 14) and closely resembled that of oestrus (fig. 7). This latter condition of complete and continuous vaginal cornification usually developed before definite xerophthalmia and growth retardation had become apparent. The cyclic changes of oestrus then became obliterated by the excessive cornification and could be followed only by the supravital method of examination of the vaginal smears (Mason and Ellison, '35).

Vaginal cornification during pregnancy. During middle and late pregnancy, in the normal rat, large nucleated cells undergoing a mucoid type of transformation are frequently observed in the vaginal smear (fig. 15). They represent the cells forming the thick mucified epithelial lining of the vagina typical of late pregnancy (fig. 16). When a mild deficiency of vitamin A existed, these cells could be observed undergoing different degrees of cornification (fig. 17). When A-depletion was more severe, this mucoid transformation was entirely suppressed and the vaginal epithelium was thickened and cornified throughout pregnancy (fig. 19).

Slightly higher levels of vitamin A appeared to be necessary for maintenance of the mucified state of the vagina during late pregnancy than for maintenance of normal vaginal smears in non-pregnant rats. It has not been possible to determine whether this was due to a greater need for A on the part of the mucified epithelium, or to a greater drainage of the maternal stores of A during late pregnancy.

B. RHYTHMICITY OF OESTRUS

The periods of complete but temporary cornification of the vaginal smears, mentioned above, did not represent a state of continuous 'heat.' The smear of the first day of such periods constituted the cornified smear of prooestrus, and pycnotic eosinophilic nuclei were frequently discernible in the cornified cells at this stage. During the mid part of these periods the cornified cells were rather indistinct in outline and tended to clump. During the last day, the cells were more discrete and more evenly distributed in the smear, bearing a closer resemblance to those of normal oestrus (fig. 7). Furthermore, as demonstrated by more than 100 mating tests with normal males, copulation occurred only during the last day of these periods. Hence, only the latter represented the period of true oestrus, or 'heat.'

With this period of true oestrus as a guide, oestrous cycles of normal duration (table 1) could be followed as long as periods of complete but temporary cornification continued to alternate with 1 or more days of partial cornification (figs. 10 to 13). Only during more advanced stages of A-deficiency, associated with definite growth retardation, did the oestrous cycle become irregular and lengthened (Mason and Ellison, '35).

TABLE 1

Showing the regularity of oestrus prior to and following abnormal cornification of A deficiency

NUMBER OF RATS IN LITTER	INITIAL BODY WEIGHT, AVERAGE	BODY WEIGHT AT BEGINNING OF VAGINAL CORNIFICATION, AVERAGE	DAYS ON EXPERIMENT BEFORE VAGINAL CORNIFICATION APPEARED, AVERAGE	BEFORE VAGINAL CORNIFICATION		AFTER VAGINAL CORNIFICATION	
				Number of oestrous cycles	Average length of cycles	Number of oestrous cycles	Average length of cycles
	gm	*gm*			*days*		*days*
5	74	188	43	26	5.22	29	5.0
4	71	169	44.5	20	5.25	24	5.0
5	70	168	47	29	5.1	8	4.75
4	68	189	51	26	5.0	29	5.0
4	66	182	56	33	4.67	22	4.6
3	64	173	41	10	5.0	24	5.58
2	62	176	53.5	13	4.23	12	4.33
3	60	180	45	10	4.8	13	5.23
3	57	163	37	11	6.3	25	5.7
4	54	138	42	12	5.4	4	5.0
Total 37				190		190	
Average[1]					5.04		5.08

[1] Based on values for individual rats.

C. REPAIR OF VAGINAL CORNIFICATION BY A-THERAPY

1. Non-castrates. The sequence of events seen in successive daily smears following A-therapy was essentially the reverse of that occurring during the onset of cornification as described above, and could be best followed in rats receiving from one-tenth to one-fifth drop of cod liver oil, daily. The latter represents the critical level of therapy for maintenance of normal smears in A-deficient rats (table 2).

The time interval required to effect repair of the vaginal epithelium (as judged by the disappearance of cornified cells of A-deficiency from the vaginal smears) varied from 1 to 13 days (table 2), depending upon the amount of A supplied.

With the higher levels of therapy, and the shorter intervals of repair, the onset of oestrus within a few days of the beginning of therapy led to a considerable range of error in determining the exact time at which repair was completed.

Mucus, which was characteristically absent from the vaginal smear after the first appearance of cornification, reappeared at about the same time that normality of the smears was established. For a week or so following the disappearance of abnormal cornification in the vaginal smears, considerable vacuolation of the nucleated epithelial cells was observed. They appeared to represent deeper cells of the epithelium which had not fully recovered from the effects of the deficiency state. Such cells were also demonstrable, at times, in the smears of normal rats. The reappearance of mucus, and the disappearance of vacuolation in the nucleated epithelial cells, did not prove to be satisfactory criteria for determining the end point of repair.

TABLE 2

Showing the repair of A-deficiency cornification of the vaginal epithelium following different levels of vitamin A-therapy

UMBER OF ESTS	AMOUNT OF COD LIVER OIL ADMINISTERED, DROPS	DURATION OF CORNIFICATION BEFORE THERAPY, DAYS			DAYS BEFORE VAGINAL SMEARS FREE OF CORNIFICATION			DURATION OF EFFECT
		Aver.	Min.	Max.	Aver.	Min.	Max.	
4	1 dr. single dose	25	20	30	4	3	5	1 to 6 days
39	2 dr. single dose	23	5	58	4	2	7	7 to 14 days
5	4 dr. single dose	46	40	53	2	2	3	15 to 20 days
14	⅒ dr. daily	19	10	28	11	9	13	Subminimal in 9 rats
15	⅕ dr. daily	18	8	24	11	8	12	Subminimal in 4 rats
12	½ dr. daily	20	15	42	5	3	8	....
6	1 dr. daily	19	16	26	4	3	6	....
30	6 dr. daily	16	..	..	2	1	3	....
6	Stock diet, daily	28	14	36	10	8	12	....

The rapidity and completeness of repair following A-therapy was well demonstrated in a group of twenty rats given

6 drops of cod liver oil, daily, beginning on the twelfth day of pregnancy; at which time the vaginal smears were extremely cornified. Within approximately 5 days cornified cells were entirely absent from the smears and large mucoid nucleated epithelial cells were present in abundance. The rapid response of the vaginal and urethral epithelia to small amounts of vitamin A is shown in figure 20.

In the tests presented in table 2, the duration of vaginal cornification prior to A-therapy varied from 5 to 58 days. Provided advanced or severe cornification (figs. 11 to 14) persisted for approximately 1 week, the previous duration of cornification had no significant effect upon the repair response of the vaginal epithelium. Other data, presented in table 2, indicate that the usefulness of the vaginal smear test as a method of bio-assay lies in the time interval between the repair of abnormal cornification and reappearance of the latter, following the administration of single doses of vitamin A-containing substances, rather than in the time interval required to effect repair.

2. *Castrates.* Forty castrate rats, in which vaginal cornification had existed for 2 to 4 weeks, were used for A-therapy tests. The dosages of cod liver oil supplied were the same as given in other tests where non-castrate rats were used (table 2), but the results were quite different. There was a marked reduction in the cornified cells of the smears, and normal nucleated cells and leucocytes appeared in large numbers. But small numbers of cornified cells usually continued to appear either constantly or at irregular intervals throughout the duration of the test, which varied from 3 to 12 weeks. There was usually no clear cut end point of repair such as observed in the non-castrate rat.

Mucus made its appearance in the smear soon after therapy was begun, and persisted throughout the period of therapy. Histologically, the vaginae were typical of the castrate condition, except for the presence of small numbers of cornified cells in the superficial layers of the thin epithelium (two- to four-cell layers), and for the presence of unusually large numbers of leucocytes forming a layer several cells deep just internal to the vaginal epithelium.

DISCUSSION

Many excellent studies of the past 10 years have offered convincing evidence that the primary effect of vitamin A-deficiency is an alteration in the epithelial cells throughout the body. The present paper adds further evidence in support of this concept, and demonstrates the reliability and usefulness of the vaginal smear test as a criterion of mild sub-xerophthalmic levels of vitamin A-deficiency.

According to the observations of Wolbach and Howe ('25, '33), Seifried ('30), and others, the fundamental effect of lack of vitamin A appears to involve an atrophy of the epithelium accompanied by, or followed by, a reparative proliferation of the basal cells. The latter, having lost their type specificity, produce a stratified keratinizing epithelium regardless of the type previously existing in that location. In recovery, each epithelium returns to its normal type (Wolbach and Howe, '33). The extent and the nature of disturbances of secretory function in epithelial cells has received very little attention.

In the vaginal epithelium, which is normally stratified and which periodically cornifies, no primary atrophy was observed after A-deficiency. But a marked increase in the proliferative activities of the epithelium was striking, as demonstrated by the increased abundance of cellular material obtained in the daily process of smearing as the deficiency progressed, and by the greatly increased thickness of the epithelium, especially in castrate rats. These observations are in accord with the increase in mitotic activity noted in the vaginal epithelium of A-deficient castrate rats by Aberle ('33 a), and with the interpretation of the metaplasia in other epithelia as observed by Wolbach and Howe ('25, '33) and others.

More striking than the increased stratification and metaplasia of the vaginal epithelium was the increased cornification of the epithelial cells, especially when cyclic changes of oestrus were occurring. In the normal rat, after the restitution of the vaginal epithelium at the approach of prooestrus, the large nucleated epithelial cells forming the internal lining of the epithelium are separated from the basal layer (stratum germinativum) by a layer of cells in which cornification occurs (stratum corneum); these two more superficial layers forming the cells of the prooestrous and oestrous smears, respectively. The obliteration of the prooestrous smear, and the increased duration of the oestrous type of smear, during the earlier phases of A-deficiency, seemed attributable to premature cornification of the cells normally forming the superficial nucleated layer and to an accentuation of the cornification normally occurring in the stratum corneum. The increased cornification of the dioestrous smear appeared to be due to less complete removal of this more cornified superficial layer of the epithelium during the degenerative changes of metoestrus, and to abnormal cornification of the deeper layers of cells, culminating in complete and continuous cornification of the smear.

That severe and prolonged cornification of the vaginal epithelium produced no permanent injury was indicated by the rapidity of repair following A-therapy, as noted also by other investigators (Evans, '28; Baumann and Steenbock, '32; Aberle, '33). The more rapid repair in this epithelium, as compared with that observed by Wolbach and Howe ('33) in other types of epithelia, appears to be due to two factors. The vaginal epithelium requires no reorganization after the influences inducing excessive cornification are removed. Furthermore, the cyclic dehiscence and regeneration of the outer layers of the epithelium associated with the oestrous cycle appeared to greatly facilitate the repair process.

The incompleteness of removal of cornified cells from the vaginal smears of A-deficient castrate rats after vitamin A-therapy has received no satisfactory explanation. It seems possible that the absence of the periodic degeneration and reorganization of oestrus, and decreased mitotic activity of the cells of the basal layer of the epithelium, associated with a more deep-seated injury induced by the previous state of

A-deficiency in the castrate epithelium, where cyclic changes of oestrus are absent, are important factors. Contrary to the belief of Hohlweg and Dohrn ('30), we feel that the castrate rat is unsuitable for tests involving the repair of vaginal cornification by A-therapy. However, in determining the time of appearance of vaginal cornification in A-deficiency, castrates prove as useful as non-castrates, but offer no advantage over the latter (Mason and Wolfe, '35).

The inability of the vaginal epithelium of A-deficient rats to produce mucified epithelial cells normally characteristic of pregnancy suggests a definite alteration in the physiologic state of the cell, rather than a deficiency of hormonal stimuli acting upon the cell. This is supported by our inability to demonstrate any significant alteration in the endocrine functions of the ovary or of the hypophysis (Mason and Wolfe, unpublished studies), and by the observations of Aberle ('33 a) that the vaginal epithelium of the A-deficient rat is quite incapable of responding to excessive administration of a placental extract which regularly induces marked mucification of this epithelium in the normal rat. It appears that, in epithelial cells deprived of adequate vitamin A, the production of glyco-proteins (mucin) is interfered with and albuminoids (keratin) appear in excess. This would suggest that vitamin A may play an important role in protein metabolism or synthesis within the epithelial cell. In the present studies, it has not been possible to determine whether the keratinizing process of A-deficiency differs, in any respects, from that normally occurring at the period of oestrus in normal rats under the influence of hormonal factors.

The observations presented in this report, and in a separate paper (Mason and Wolfe, '35), clearly demonstrate the reliability of vaginal cornification as an index of early stages of vitamin A-deficiency. These findings are in disagreement with those of Parkes and Drummond ('26), Coward ('29), Coward, Morgan and Dyer ('30) and Thatcher and Sure ('32), as discussed more fully elsewhere (Mason and Ellison, '35).

As a method for the biological assay of vitamin A, the vaginal smear test seems best applicable to the measurement of the interval elapsing between the repair of, and the return of, vaginal cornification following administration of single doses of vitamin A-containing substances. Unlike the other criteria of A-deficiency (xerophthalmia and growth plateau), vaginal cornification is not significantly influenced by other factors (differences in inherent growth capacity of the test animal, general debility, extraneous causes of ocular irritation and infection) which may alter the reparative response when measured by other methods of assay. The lesser degree of A-depletion required in the test animal, and the utilization of the same animal for repeated tests at intervals of a few weeks, offer additional advantages.

As far as we can ascertain, no attempt has yet been made to determine whether lack of vitamin A produces increased cornification of the vagina in the human. The evidence at hand would suggest that vitamin A is essential for the maintenance of a normal healthy state of this epithelium. Of interest in this latter connection are recent observations of Dr. J. W. Simpson, of the Department of Obstetrics and Gynecology of this school (personal communication), dealing with the efficacy of a high intake of vitamin A in the treatment of senile vaginitis, especially in patients in whom the dietary history suggested a low intake of this factor. Not only were the clinical and macroscopical manifestations much more rapidly improved than by the more usual methods of treatment, but small portions of the vaginal epithelium removed by biopsy, at intervals during treatment, showed marked histologic evidences of repair.

The usefulness of the vaginal smear test in detecting early stages of vitamin A-deficiency in the rat, and in permitting study of the injury induced by mild levels of A-deficiency undetectable by other pathognomonic signs, is demonstrated in a separate study (Mason, '35).

SUMMARY AND CONCLUSIONS

1. The changes occurring in the dry stained vaginal smears of castrate, non-castrate, and pregnant rats during the development of vitamin A-deficiency, and during repair of the deficiency state, are described. These observations are based upon the study of more than 52,000 vaginal smears.

2. Lack of A results in an early cornification of the nucleated epithelial cells typical of the prooestrous smear, and in an accentuation of the process of cornification normally occurring at oestrus, resulting in the appearance of completely cornified smears during several successive days. The last day of such periods represents the stage of true oestrus, or 'heat.'

3. There also occurs a progressive cornification and an increased proliferation of the nucleated epithelial cells of the dioestrous type of vaginal smears, associated with a corresponding reduction in the relative number of leucocytes, culminating in continuous and complete cornification of the smears before xerophthalmia and growth retardation become apparent.

4. As a result, the cyclic changes of oestrus are represented by alternating periods of partial (metoestrus and dioestrus) and complete cornification (prooestrus and oestrus) of the vaginal smears. Until the two types of smear merge into a state of complete and continuous cornification, oestrous cycles of normal duration can be followed by the dry stained smears.

5. Mucus disappears from the smear at the onset of cornification and reappears during the early stages of repair.

6. Except in castrate rats, daily administration of one-tenth to one-fifth drop of cod liver oil usually effects repair of severe vaginal cornification during the second week of therapy, and represents the approximate minimal level of therapy for maintenance of normal vaginal smears in A-deficient rats. With larger doses of vitamin A, cornified cells disappear from the smear within 1 to 2 days.

7. Small numbers of cornified cells continue to appear, in the vaginal smears of castrate rats, even after long periods of vitamin A-therapy, invalidating the use of such rats for assay tests by the smear method.

8. The usefulness of the vaginal cornification test as a method of bio-assay, and as a criterion of mild levels of vitamin A-deficiency, is discussed.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures are photographs of vaginal smears of the rat. × 100.

Stained in haematoxylin and eosin.

1 Normal dioestrus. Considerable mucus with many leucocytes and a few nucleated epithelial cells.

2 Early cornification during dioestrus. A few large cornified cells intermingled with leucocytes and large, swollen, nucleated epithelial cells.

3 Early cornification during dioestrus. Approximately equal numbers of completely cornified cells and nucleated cells, some of which exhibit early stages of cornification. Mucus thin and watery.

4 Early cornification during transition from late dioestrus to early prooestrus. Shows many large, swollen nucleated epithelial cells with faintly acidophilic or faintly basophilic nuclei surrounded by a ring of faint cytoplasm. Few leucocytes are present. The mucus is becoming thin and watery. The following day the smear resembled that of figure 9.

5 Normal prooestrus, showing large basophilic nucleated epithelial cells.

6 Normal prooestrus (late), showing smaller nucleated epithelial cells with pycnotic nuclei and faintly acidophilic cytoplasm, as frequently observed during the transition period between prooestrus and oestrus.

7 Normal oestrus, showing the large discrete, flaky cornified cells typical of this stage.

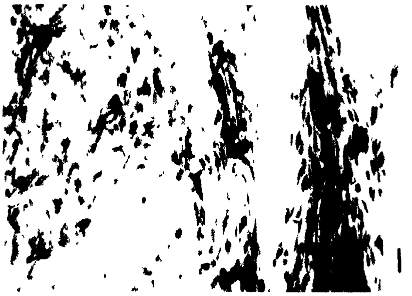
8 Normal metoestrus (late), showing large numbers of basophilic nucleated epithelial cells and leucocytes, together with numbers of pale cornified elements persisting from the preceding stage of oestrus.

9 Atypical prooestrus characteristic of early stages of cornification. The large basophilic nucleated epithelial cells typical of this stage (fig. 5) have undergone extensive swelling and cornification. Pycnotic nuclei are visible in many cells.

10 Moderate cornification appearing during dioestrus. The majority of nucleated epithelial cells show varying degrees of cornification. Abundance of leucocytes, but no mucus.

VAGINAL SMEAR VITAMIN A-DEFICIENCY
K. E. MASON AND E. T. ELLISON

PLATE 1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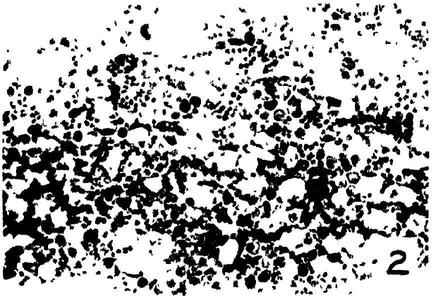
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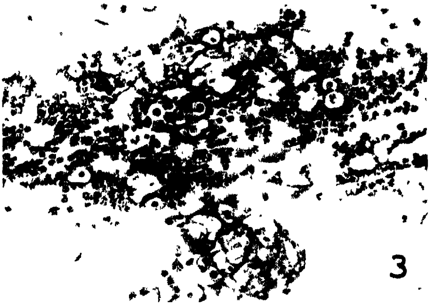
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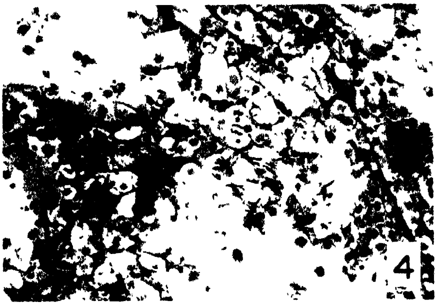
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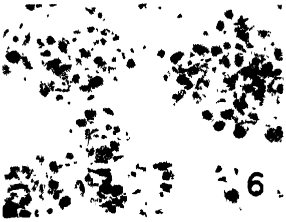
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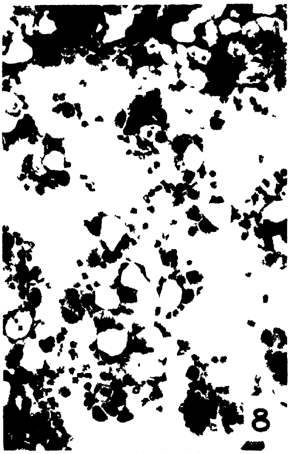
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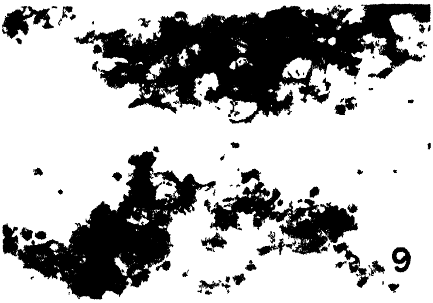
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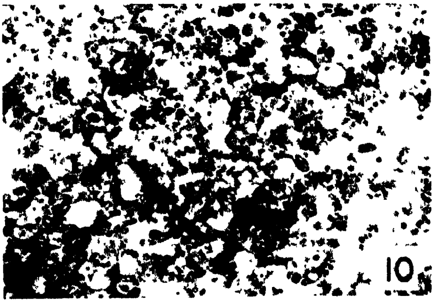
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PLATE 2

EXPLANATION OF FIGURES

Figures 11 to 15, 17 and 18 are photographs of vaginal smears of the rat. × 100.

Figures 16, 19 and 20 are photographs of sections of the vagina of the rat. × 100.

Stained in hematoxylin and eosin.

11 Advanced cornification. Large numbers of cornified cells and leucocytes; very few nucleated epithelial cells.

12 Advanced cornification. Similar to figure 11, except for larger proportion of cornified epithelial cells.

13 Extreme cornification. Only a few leucocytes and extruded nuclei of degenerated nucleated epithelial cells are interspersed among the large completely cornified cells.

14 Extreme cornification. Representing stage of complete cornification. Resembles smear of normal oestrus (fig. 7), except for the extreme paleness of the cornified cells.

15 Vaginal smear on seventeenth day of pregnancy, from rat receiving one-fifth drop of cod liver oil, daily. This level of therapy was adequate to repair the previous vaginal cornification, as demonstrated by the presence of the large mucified epithelial cells which are identical with those commonly observed in normal rats during the second half of pregnancy.

16 Showing the tall mucified type of vaginal epithelium of the normal rat at the end of pregnancy.

17 Vaginal smear on fifteenth day of pregnancy, from rat receiving subminimal level of A-therapy (one-tenth drop of cod liver oil, daily). Such smears are frequent when mild, chronic deficiency of A exists during pregnancy. The deeply stained elements are mucified nucleated epithelial cells similar to those of figure 15. The grayish masses represent many nucleated epithelial cells undergoing all degrees of cornification. A thin, watery mucus is usually present in such smears.

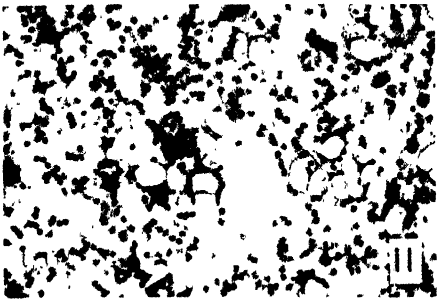
18 Vaginal smear of castrate rat which, after 20 days of continuous cornification, received daily administration of one-fifth drop of cod liver oil for 43 days, and of 2 drops of cod liver oil for 32 more days. Cornified cells continued to persist in the smear. This smear was taken on the seventy-fifth day of therapy.

19 Thick cornified stratified epithelium of vagina (left) and of urethra (right), from A-deficient rat at the end of pregnancy, during which cornification of vaginal smears was complete and continuous.

20 Vaginal epithelium (left) and urethral epithelium (right) from rat which had shown severe vaginal cornification for 18 days after which one tenth drop of cod liver oil was administered, daily, during the 4 days preceding autopsy. Compare with figure 19, representative of the condition prior to therapy, and note the marked reduction in thickness of both epithelia, due to sloughing of the superficial layers of cornified cells.

VAGINAL SMEAR VITAMIN A-DEFICIENCY
K. E. MASON AND E. T. ELLISON

PLATE 2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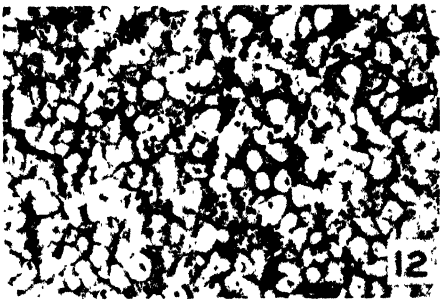
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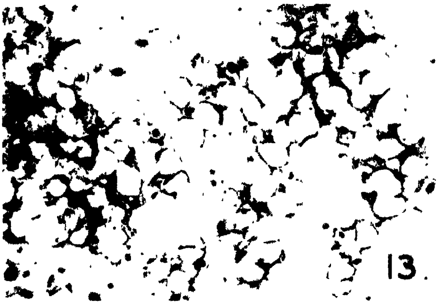
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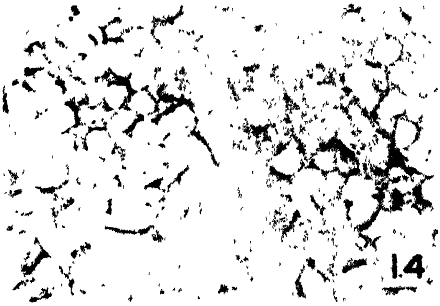
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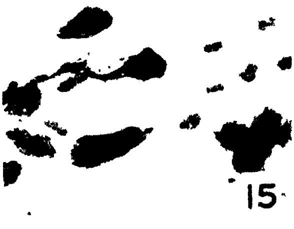
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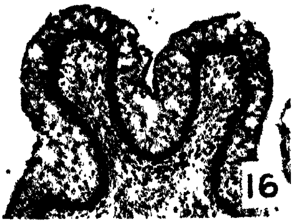
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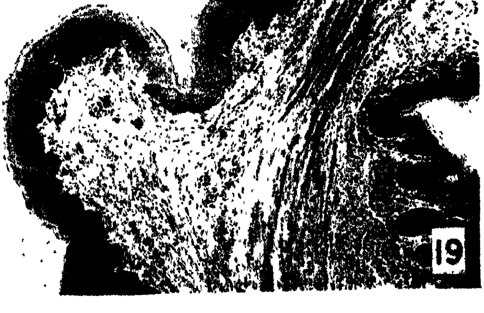
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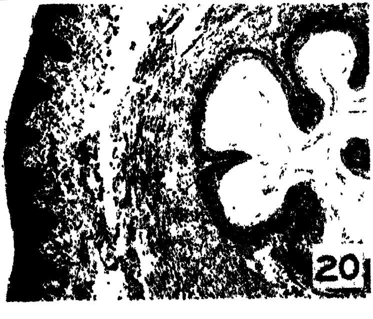
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PROCEEDINGS OF THE SECOND ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

MINUTES OF MEETING OF AMERICAN INSTITUTE OF NUTRITION, DETROIT, APRIL 10, 1935

The second annual meeting of the American Institute of Nutrition was held in Detroit at the Masonic Temple on April 10, 1935, according to printed program sent to all members. Seventy-six members and 203 guests registered and approximately 400 to 500 people were in attendance during the entire program.

President John R. Murlin presided at the morning scientific session and business meeting. Vice-President E. F. DuBois presided during a part of the afternoon scientific session. The meeting started promptly at 9.30 A.M. and for the most part each speaker was thoughtful enough to confine his comments rigidly to the time allotted to the presentation of his paper thus making it possible to start the business meeting at 11.30 A.M. according to schedule and to close promptly at 1.00 P.M. The second scientific program was conducted in the same expeditious and satisfactory manner. All twenty papers listed on the program were presented.

The business meeting was called to order by President Murlin. The reading of the minutes of the preceding meeting was dispensed with since they had been published in The Journal of Nutrition and could be read by all. The president announced that any comments on the minutes as published could be sent in writing to the secretary.

The report of the Treasurer was then read and accepted. Harold B. Pierce and Harold E. Himwich were asked to serve as auditors. After examination of the treasurer's report they declared it correct.

The annual assessment for 1935–1936 was fixed at $1.00 by unanimous vote of the membership.

The committee appointed to consider changes in the constitution[1] of the American Institute of Nutrition submitted their report. After much discussion of the various items the following changes in the by-laws were adopted (the changes are italicized):

Article I. Membership. Section I. *Membership:* *There shall be two classes of members, members and emeritus members. The number of members shall be limited to 250; exclusive of emeritus members.*

Section II. Eligibility for membership: *Members.*—Qualified investigators who have independently conducted and published meritorius original investigations in some phase of the chemistry or physiology of nutrition *and who have shown a professional interest in nutrition for at least 5 years* shall be eligible for membership in the society. *Emeritus members.—Members in good standing who have reached the age of 65 years shall become emeritus members. A member in good standing and for sufficient reason may by vote of the council be made an emeritus member. Emeritus members shall be entitled to vote but not hold office.*

(Change the designation of Sections 3 and 4 to 4 and 5 respectively.)

Article VI. Dues: *The dues shall be the annual cost of subscription to The Journal of Nutrition for members plus an* annual assessment *which* shall be determined by majority vote at the annual meetings, upon recommendation of the council, and shall be due within a month after the annual meeting. *Emeritus members are not required to pay assessments nor to subscribe to The Journal of Nutrition.*

(NOTE: The dues will be $4.00 plus the assessment for those who have already subscribed to the journal for the year 1935. This will place the journal year from July 1st to June 30th. Thereafter the dues will be $8.00 plus the assessment. For members who do not now subscribe to the journal the dues will be $8.00 plus the assessment for the year 1935–1936.)

Article VII. Section 2. (Beginning with the subsection lettered 'A.') Subsection A. *The editorial board shall consist of twelve regular members and a managing editor.* B and C (the present subsections now lettered A and B) the managing editor shall be chosen by the editorial board to serve a period of 5 years. *He shall be eligible for re-election.*

The next item of business was the election of officers. Franklin C. Bing, Ray G. Daggs and Walter C. Russell were asked to act as tellers for this election.

The nominating committee for 1935, composed of E. B. Forbes, Henry A. Mattill (appointed to replace A. W. Rowe, deceased), H. S. Mitchell, W. S. Sperry and Harry Steenbock, chairman, proposed the following nominees:

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[1] Original Constitution—see J. Nutrition, vol. 7, no. 5 and supplement, 1934.

Officers		*Editorial Board* (4 to be elected)
President	J. R. Murlin	G. R. Cowgill
Vice-President	E. F. DuBois	H. B. Lewis
	F. C. Koch	I. G. Macy
Secretary	I. G. Macy	E. M. Nelson
Treasurer	W. M. Boothby	L. S. Palmer
	G. R. Cowgill	W. C. Rose
Councilor	L. A. Maynard	A. T. Shohl
	G. E. Cullen	A. H. Smith

Doctor Steenbock, in discussing the report, stated that the nominating committee had a considerable number of ballots cast in arriving at its list of nominees. The committee appreciated that there were certain questions of precedence which might be involved and was exceedingly careful in arriving at its conclusion. Nevertheless, it saw fit to nominate Doctor Murlin as president in face of his protest against the possible establishment of a precedent of re-electing officers from year to year because it wanted to express in some small measure appreciation for the excellent work he has done for the society. In the case of the secretary, the committee felt that on account of the familiarity of the present secretary with the work involved it would be to the credit and advantage of the society to have her re-elected. Although the president called for nominations from the floor it was moved and seconded that the nominations be closed. Furthermore, it was moved and seconded that the secretary be instructed to cast a unanimous ballot for Doctor Murlin as president and the president in turn was asked to cast a unanimous ballot for Icie G. Macy as secretary. The tellers reported the following election of officers by ballot:

a) Members of Council:
    E. F. DuBois, Vice-President
    G. R. Cowgill, Treasurer
    L. A. Maynard

b) Editorial Board:
    W. C. Rose
    A. H. Smith
    G. R. Cowgill
    H. B. Lewis

In discussing the new amendment on dues Paul E. Howe suggested that it be a matter of choice if a member takes one or two volumes of The Journal of Nutrition at a time. It was also decided that the society should collect the dues (including the annual assessment and the journal fee) and pay to the journal.

The opinion was expressed that it was quite unfair for a member to subscribe to the journal and then turn it over to the library in an institution. The society loses because members pay $4.00 per volume and non-members $5.00.

The council which had met at the Hotel Statler on Tuesday evening, April 9th, reported the following names as qualified for membership in the American Institute of Nutrition:

Leah Ascham
James Harold Austin
Joseph H. Axtmayer
Charles E. Bills
L. Jean Bogert
Alfred Bosworth
W. W. Braman
Margaret Hessler Brookes
Thomas B. Cooley
Gerald J. Cox
Robert Gibson
Estelle E. Hawley
V. G. Heller
C. A. Hoppert
Percy R. Howe
Rebecca Hubbell
J. F. McClendon
E. W. McHenry
Ade T. Milhorat
R. C. Miller
Elsa R. Orent
Herbert Pollack
Myra Sampson
Frederick H. Scharles
Philip A. Shaffer
Henry J. Spencer
F. W. Sunderman
Dee Tourtellotte
Harry M. Vars
James Waddell
Bruce Webster

It was moved by Paul E. Howe and seconded by E. F. DuBois that the candidates approved by the council be elected to membership in the American Institute of Nutrition. The motion was carried unanimously.

The chairman announced the resignation of Robert C. Moulton. E. B. Forbes moved that Doctor Moulton be continued as an emeritus member. This was seconded by E. F. DuBois. The motion carried.

In accordance with the by-laws the chairman appointed the following nominating committee for 1936: Henry C. Sherman, chairman, R. Adams Dutcher, Fred A. Hitchcock, Charles G. King and Helen T. Parsons.

The chairman announced that the council recommends a continuance of the policy for the present at least, of holding the meeting of the American Institute of Nutrition one day in advance of the Federation meeting. There seems to be no objection to this arrangement.

The noon luncheon was attended by 141 members and guests.

R. M. Bethke moved that the following resolution be recorded in the minutes:

> That the secretary be instructed to write the Convention Bureau, the Masonic Order and the Local Committee and express the thanks and appreciation of the American Institute of Nutrition for the splendid accommodations provided for the meetings and all other arrangements that added to the comfort and pleasure of the members and their guests.

At the closing of the meeting the president announced the untimely death of Allen Winter Rowe of Boston during the past year. The members stood for a moment in silence in token of respect for Doctor Rowe.

ICIE G. MACY,
Secretary.

PROGRAM OF PAPERS PRESENTED

ABSTRACTS

Experiments with a new type of antirachitic substance. Lester Yoder, B. H. Thomas and Malcolm Lyons, Iowa Agricultural Experiment Station, Ames Iowa.

Recently Yoder[1] reported that an antirachitic derivative, namely, cholesterilene sulfonic acid, can be prepared from cholesterol without resorting to irradiation. We have found in working with this derivative and certain of its salts that their effects on intestinal reaction, iron reduction and calcification are very similar to those of viosterol.

The calcium salt of cholesterilene sulfonic acid has been repeatedly prepared and has been found to possess the same constant composition and antirachitic potency. Sulfonic acids prepared in the same manner from a variety of synthetic alcohols have failed to exhibit any antirachitic activity.

In view of the wide differences in the efficacy of cod liver oil and viosterol as antirachitics for chicks, calcium cholesterilene sulfonate was compared with these commonly employed supplements. Two such tests have demonstrated that chicks are able to utilize calcium cholesterilene sulfonate more efficiently than cod liver oil and much more so than viosterol. For example, the tibiae of chicks fed rations containing 2 rat units of cod liver oil or 50 rat units (Steenbock) of viosterol per 100 gm. of ration contained practically the same percentage of ash as those of the negative control group; namely, 28.7 per cent. However, chicks similarly fed rations containing 2 and 50 rat units (Steenbock) of calcium cholesterilene sulfonate yielded bones which ashed practically the same as the positive control group; namely 47.3 per cent.

[1] Lester Yoder, Science, vol. 80, p. 385, 1934.

Variability of vitamin D response with temperature of environment. D. Tourtellotte (introduced by M. S. Fine) and W. Bacon (by invitation), Research Laboratories, General Foods Corporation, Battle Creek, Michigan.

The routine use of a standardized cod liver oil as a control for vitamin D assays during a 2-year period has shown the variability in vitamin D response with temperature of environment and indicates the necessity for air-conditioned animal quarters where precise vitamin D assays are to be made. Although some nutrition laboratories have such air-conditioned rooms, no published data showing the variability in assay results due particularly to uncontrolled summer temperatures have appeared.

The vitamin D estimations were made by the Bills 5-day line test modification which possesses the advantage of speed and reliability over other existing vitamin D assay procedures. Variables due to dietary constituents have been reduced to a minimum by the purchase of a year's supply of grains after careful preliminary tests as to their suitability. Mean temperatures above 80°F. during the rachitogenic and test periods caused an apparent three- to four-fold fluctuation in vitamin D potency of a standard control cod liver oil. The error of the assay was due to an increased severity of rachitogenesis during periods of elevated temperatures as shown by the development of standard line test rickets in 11 to 14 days as against the specified period of 21 days. Reducing the calcium: phosphorus ratio of the diet compensated for the increased rate of rachitogenesis.

The comparative antirachitic efficiency of irradiated milk, yeast milk and cod liver oil. R. M. Bethke, W. E. Krauss, P. R. Record (by invitation) and O. H. M. Wilder (by invitation), Departments of Animal Industry and Dairy Husbandry, Ohio Agricultural Experiment Station, Wooster.

A comparison was made with chicks of the antirachitic efficiency of irradiated milk, milk produced by the feeding of irradiated yeast to cows, and cod liver oil, on a rat unit basis.

The dried whole milks were first assayed for vitamin D with rats and then compared on the rat unit basis with chicks. Equivalent amounts of milk solids, either in the form of dried skim milk or dried whole check milk, were included in the negative and positive control chick rations. Ash analyses of the tibiae were made at the end of the fifth or sixth week.

The data showed that it required more than ten times as many rat units of vitamin D in the form of yeast milk than in the form of irradiated milk to produce the same degree of calcification. Vitamin D from irradiated milk and cod liver oil were equally efficient.

Tho same milks (irradiated and yeast) were found to be equally efficient for the cure of rickets in infants by Gerstenberger, Horesh and Van Horn, when fed on an equivalent rat unit basis. These observations indicate that the infant and chick vary greatly in their utilization of vitamin D from different sources.

The antirachitic efficiency of irradiated milk, yeast milk, cod liver oil, and irradiated cholesterol. R. W. Haman (by invitation) and H. Steenbock, Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.

The primary object of our experiments was to compare the antirachitic efficiency of a unit of vitamin D from irradiated milk with that from so-called yeast milk produced by the feeding of irradiated yeast to cows. In line with previous work the milks were standardized with rats and the comparison of efficiency was made with baby chicks. The milks in the first experiments were fed incorporated in a rachitogenic ration. Later butter fat from the milks was used instead. Bone ash analyses were made after 5 weeks.

The data obtained revealed that for the chicken a unit of vitamin D from irradiated milk was approximately ten to fifteen times as efficient as a unit from yeast milk. Irradiated milk proved to be as efficient as cod liver oil. Furthermore our data confirmed the observation of Dr. James Waddell that irradiated cholesterol had the same antirachitic efficiency as cod liver oil.

In view of our data it is evident that there is an urgent need for correlative studies with infants. So far as data with infants are available in the literature, it is evident that the difference in efficiency of cod liver oil and irradiated milk as compared with yeast milk is not the same as we found it to be with chickens.

The healing of rickets in rats on a diet containing negligible amounts of calcium and vitamin D. James H. Jones and B. N. E. Cohn (by invitation), Departments of Physiological Chemistry and Orthopedic Surgery, School of Medicine, University of Pennsylvania, Philadelphia.

Young rachitic rats, which were transferred to a synthetic diet of normal phosphorus content but containing at most only traces of either calcium or vitamin D, showed a marked decrease in food consumption and developed tetany within 18 to 48 hours. After 48 hours the animals were divided into three groups: group I, sacrificed; group II, continued on synthetic diet; group III, given irradiated ergosterol. Serum calcium and phosphorus of group I were about 6 and 10 mg., respectively, and the 'line test' indicated slight healing. The food consumption of groups II and III increased after 48 hours and tetany disappeared. Serum calcium increased while phosphorus remained high. After a few weeks both groups of animals showed complete healing of the rickets. The shafts of the long bones were very fragile with numerous spontaneous fractures. The absolute amount of ash in the femora of the animals of groups II and III appeared to be slightly more than in those of group I. Since healing of the rachitic lesions and the possible slight increase in femur ash occurred on a diet containing negligible amounts of calcium the bone salts must have been transferred from calcified portions of the skeleton to the more rachitic parts. Furthermore, as healing took place in the animals not receiving viosterol apparently vitamin D is not essential for this metastasis.

The influence of some commonly used salt mixtures upon growth and bone development of the albino rat.[1] Lafayette B. Mendel, Rebecca B. Hubbell (by invitation) and Alfred J. Wakeman (by invitation), Biochemical Laboratory of the Connecticut Agricultural Experiment Station, New Haven.

In order to devise a salt mixture suitable for use with rapidly growing rats, a study was made of four well-known preparations. Male rats in groups of ten were placed at weaning on diets of purified foods in which the salt mixture and the level at which it was fed were the variables.

Comparisons of the rate of growth from 60 to 200 gm., and of the ash of the dry, fat free femurs showed that the mixtures are not interchangeable at any of the six levels studied. Rats given 4 per cent Osborne-Mendel or McCollum salts showed the same daily gain (5.2 gm.), but the bone ash was quite different, being 60.8 and 57.9 per cent, respectively. With 2 per cent Steenbock salts, the gain was 4.0 gm. and the bone ash 50.1 per cent, whereas with Osborne-Mendel salts, the animals gained 5.8 gm. per day and attained a higher bone ash (51.8 per cent) in spite of the more rapid growth.

If to 1 per cent of any of the four mixtures enough $CaCO_3$ was added to raise the calcium to the level in 5 per cent Osborne-Mendel salts growth was rapid in all cases (5.0 to 5.8 gm. daily) and calcification of the femurs occurred to about the same degree (57.4 to 58.9 per cent).

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[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

The influence on iron assimilation of some of the elements in groups 1 and 2 of the periodic system. Seymour W. Kletzien, State Institute for the study of Malignant Disease, Buffalo, New York.

In the past year evidence has been presented by the author showing the definitely inhibitive effect on iron assimilation of calcium carbonate added to an otherwise complete diet. These studies have been extended since the presentation of the initial report to include other calcium salts as well as the carbonates of sodium, potassium, beryllium, strontium, and barium. The data reveal that the role of these elements in iron assimilation is distinct, if not unique, and that they fall according to their ability to facilitate or inhibit iron assimilation into either group 1 or 2 of the periodic system.

The significance of this work aside from its more abstract or purely scientific aspects lies in its direct application to our present day nutrition problems among which the studies in reproduction, anemia, and respiratory disease figure so conspicuously. Further, it appears to throw light on those problems relating to iron and beryllium injections in experimental tuberculosis, and lastly, it affords an apparent answer as to why we are born rich in iron and poor in calcium.

Indispensability of zinc in the nutrition of the rat. Frank E. Stirn (by invitation), C. A. Elvehjem and E. B. Hart, Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.

Data are presented substantiating our former work which demonstrated that zinc was essential in the nutrition of the rat. We had shown that zinc was required for normal growth. However, about the same time Newell and McCollum reported no differences in animals on zinc low and zinc fortified diets with the exception of a variability in the animal's zinc content. In continuing our work the ration was further improved by lowering its zinc content and increasing its growth promoting properties by supplying the water soluble vitamins from milk serum. On this ration the animals which did not receive zinc consistently showed marked retardation of growth as compared to litter mates on the same ration to which zinc had been added. No specific pathological changes were exhibited by the animals on the zinc low ration except that they became hyper-irritable and developed an abnormal fur coat. That the ration was complete in every respect, with the exception of zinc, was shown by the fact that the animals which received this ration with added zinc were apparently normal. Food intakes showed that the zinc low animals were less efficient converters of ration into body weight and also that they consumed appreciably less ration. This work demonstrates conclusively that zinc is an essential element for the normal nutrition of the rat.

The relation of selenium to a toxicosis produced by ingestion of wheat. E. M. Nelson and D. Breese Jones, Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture.

Four years ago the attention of Dr. Henry G. Knight, chief of the Bureau of Chemistry and Soils, was directed to the fact that a disease of obscure origin occurred in farm animals, and furthermore, that wheat and other grains grown in the same locality where the disease occurred produced toxic symptoms when fed to laboratory animals. Extensive research in the U. S. Department of Agriculture has established that the toxic nature of such wheat is due to the presence of selenium. Experimental evidence is offered showing that this toxic selenium is associated with certain proteins.

Utilization of inulin by the white rat. Howard B. Lewis and Adelaida Bendana (by invitation), Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

The problem of the utilization of inulin was approached through a study of the growth curves of young white rats on a diet adequate except in calorific value. Control animals 60 to 70 gm. in weight, on a diet supplying approximately 12 cal. daily, with either fructose or sucrose as a source of carbohydrate, showed growth curves which were essentially flat. Animals on the basal diet plus 1.5 gm. of inulin (chicory) daily showed much better growth than the controls. However, rats given the basal diet plus 1.5 gm. of either sucrose or fructose daily showed a markedly superior growth as compared with the rats fed inulin. Thus, although inulin may be utilized to some extent, its nutritive value is not comparable to that of either fructose or sucrose.

In a number of experiments in which inulin entirely replaced either sucrose or fructose, all except four rats in one series died within 15 days. Death was probably due to marked distention of the large intestines, with changes in pressure relations caused by intestinal fermentation of the inulin.

An attempt was also made to determine whether any difference exists between inulins of different botanical origins by following growth curves of rats on the basal diet plus 1.5 gm. daily of either dahlia or chicory inulin. No evidence of a difference in the nutritive value was obtained.

Regression as a tool in the biological assay of vitamin A. Pearl P. Swanson and Gladys H. Timson (by invitation), Nutrition Laboratory of the Foods and Nutrition Department, Iowa State College, Ames.

Within limits, gain in weight of depleted rats is a linear function of the quantity of vitamin A fed. The proportionality between dose and response holds after gains induced by graded levels of vitamin feeding have been translated into international units by means of a 'curve of response' fitted by the method of least squares.

Significant correlations have been found between the increase in body weight during the test period and the age and weight of the animal at the end of the depletion period, the quantity of food consumed during this interval, and the length of the depletion period.

By means of a regression equation involving the above factors and calculated from data on 577 female rats, the mean response of groups of rats to 0.5 International unit has been successfully estimated. Information concerning expected behavior of individual A-depleted rats ready for supplementary feeding may be gained by the same regression. Those animals whose response can thus be estimated as erratic are eliminated at this stage, whereby, increased reliability of results is obtained with a decreased number of animals.

The influence of vitamin C level upon resistance to diphtheria toxin. C. G. King and M. L. Menten (by invitation), University of Pittsburgh.

Guinea pigs under controlled conditions and receiving definite daily quantities of vitamin C at abundant, protective, and sub-protective levels were given subcutaneous injections of standardized diphtheria toxin in 0.1, 0.3, 0.5, and 1.0 M.L.D. quantities. The survival time was shortened about 50 per cent and loss in body weight was more severe when the animals were partially depleted of their vitamin C reserves, without showing external signs of scurvy. Contrasts in degree of hemorrhage and necrosis at the sites of injection were more marked than weight and survival differences. It is evident from the study that there is a wide zone of vitamin C deficiency, without the appearance of scurvy, where physiological processes are subnormal and the animal is more sensitive to injury from bacterial toxin. Histological examination of the tissues from a large number of experimental animals has confirmed the differences in degree of injury observed macroscopically, and has also indicated a close relationship to certain human pathological conditions of particular interest is the relationship to arteriosclerosis.

The effects of the plane of protein intake on the utilization of food energy and protein. E. B. Forbes, R. W. Swift, Alex Black (by invitation) and O. J. Kahlenberg (by invitation), Institute of Animal Nutrition, Pennsylvania State College.

The effects of four planes of protein intake were studied in a 10 weeks growth, metabolism and body analysis experiment on forty-eight albino rats.

The diets contained 10, 15, 20 and 25 per cent protein, and were made equicaloric by compensatory change in carbohydrate and fat.

The rats were selected as twelve groups of four individuals, each group being of one sex and of the same litter, the rats in each such group receiving the same energy but different protein intake.

Growth increased with increase in protein to 20 per cent, but the 25 per cent group grew essentially the same as did the 20 per cent group.

With increased protein but constant energy intake the digestibility of the protein increased slightly, and linearly; urinary nitrogen increased at an increasing rate, and protein of body gain increased at a decreasing rate.

With increased protein but constant energy intake the feces energy decreased as the urinary energy increased, the metabolizable energy therefore being practically constant. The energy of the body gain and of the protein gain increased materially but of the body fat there was not significant gain.

With increased protein but constant energy intake the heat production diminished materially, which is considered to be due to the removal from the circulation of heat-stimulating nutrients, as results of improved nutritive balance and increased synthesis of body substance.

The variability of the heat production of normal human subjects. Joseph Berkson (by invitation), Halbert L. Dunn (by invitation) and Walter M. Boothby, Section on Biometry and Medical Statistics and Section on Clinical Metabolism, The Mayo Clinic, Rochester, Minnesota.

A statistical analysis of the observations of heat production in relation to age, stature, and body weight has been made in a large series of normal human subjects (641 males, 776 females). These included series of successive observations made on the same individual (intra-individual) as well as single observations made on successive individuals (inter-individual).

Particular stress is laid in this presentation on a study of the normal variability of heat production both intra-individual and inter-individual. 1) Observations of heat production made on the individual correspond in general to the statistical bell-shaped 'normal curve.' When numerous observations are made on the same subject the concept of random variation is applicable to them. 2) We found that the intra-individual variability accounts for a large part of the variation observed in single observations made on numerous subjects.

Water balance. L. H. Newburgh and Margaret W. Johnston (by invitation), Department of Internal Medicine, University of Michigan, Ann Arbor.

The weights of the following items need to be known in order to deal quantitatively with water balance: Sources: 1) ingesta; 2) endogenous, a) oxidation, b) preformed. Losses: 3) urine; 4) stool; 5) vapor. In order to calculate water of oxidation, preformed water and water vapor, the subject is fed a constant diet of known composition and low in carbohydrate for at least 3 days preceding the period and continued during the study. Under these circumstances, he will metabolize the carbohydrate of the diet. The protein metabolism is calculated from the N excretion. The subject is instructed to adjust the clothing so that he will be comfortable, i.e., he is neither too warm nor too cool.

Since I.W. (vapor) = I.L. (insensible loss of weight)—(CO_2–O_2), the weight of I.W. can be calculated as soon as the fat metabolized is known. This last value may be computed by means of the following equation:

$$F = \frac{\text{I.L.} - (2.12\,\text{CHO} + 10.56\text{N})^{1}}{3.78}$$

The total CO_2 and O_2 of the metabolic mixture may now be calculated and I.W. derived.

The water of oxidation is calculated from the metabolic mixture and preformed water derived by comparing the diet with the metabolic mixture.

The water of the ingesta, urine and stool, is obtained according to standard procedures.

[1] Lavietes, P. H.: Journal of Clinical Investigation, 1935, page 57.

The inter-relationship between nitrogen, calcium and phosphorus metabolism of pre-school children. Jean E. Hawks (by invitation), Merle M. Bray (by invitation), Marjorie G. Olsen (by invitation) and Marie Dye, Home Economics Section, Michigan Agricultural Experiment Station, Michigan State College.

During 24 consecutive days, following a 20-day preliminary period, four normal pre-school children received a constant diet containing 3 gm. of protein per kilogram of body weight. For the next 24 days they had the same diet except that the protein was increased to 4 gm. per kilogram. Calcium and phosphorus remained constant. Food, feces and urine were analyzed for nitrogen, calcium and phosphorus.

Values for the fairly constant nitrogen retentions followed intake variations closely on the first diet and on the last 15 days of the second diet, but showed little correlation during the first 9 days following the diet change. On the second diet, nitrogen retentions were higher, calcium retentions slightly lower and phosphorus retentions slightly higher.

The values for nitrogen, calcium and phosphorus retentions, expressed as retention ratios, indicate an inter-relationship between the utilization of these minerals. The increased diet protein caused a greater nitrogen retention which possibly carried with it a slightly greater proportion of phosphorus than the nitrogen retained on the lower protein diet. It also lowered the Ca/P retention ratio suggesting an increased growth of tissue other than bone. It may have limited the amounts of calcium and phosphorus available for bone growth by depositing absorbed phosphorus with nitrogen. Thus it may have altered metabolic processes.

The effect of various dietary principles on milk production. Ray G. Daggs, Department of Vital Economics, University of Rochester, Rochester, N. Y.

The method for the study of lactation in rats, reported by title at the 1934 meeting of the American Institute of Nutrition, is dependent upon two growth constants derived from plotting the logs of the litter weights from the fourth to the seventeenth day of life against time in days. Two straight lines are obtained, the break occurring at the tenth day. By combining the two slope constants a relative lactation index is obtained. The relative lactation value of different diets is shown by these indices.

By using this method the lactation promoting factor, as determined from some 300 experiments, was found to be present in liver, egg, beef round, kidney, alcohol-water extract of liver, diets of higher protein level (liver and casein), water extract of autolysed liver or egg, Witte's peptone, blood fibrin, lactalbumin, cystine and a combination of 1 part cystine, 1 part glycine and 1 part glutamic acid fed with only 12 parts of casein and the other constituents of the basal diet. This last mixture which simulates glutathione gave the best results, although cystine alone as a supplement showed a remarkable effect. It is believed that cystine or glutathione acts in some way as a mammary stimulant.

Two human cases to whom the amino acid mixture was fed showed favorable results.

Continuous nitrogen and mineral balances during pregnancy, puerperium and lactation. Helen A. Hunscher, Frances Cope (by invitation), Helen R. Sternberger (by invitation), Betty Nims Erickson (by invitation) and Icie G. Macy, Children's Fund of Michigan, Detroit, Michigan.

Continuous metabolic balance studies were made from the one-hundred and thirty-fifth day of the fourth pregnancy to the fifty-third day of lactation including the puerperium, on a healthy woman under our constant observation for 6 years.

The diet voluntarily chosen abounded in minerals, protein and vitamins from fruits, vegetables and 2 quarts of milk each containing 150 Steenbock units vitamin D (Vitex). Data on the storage of seven acid-base mineral elements and N are reported for twenty-eight periods of 5 days each during pregnancy and eight periods during lactation. The mean daily food intake during pregnancy, Ca, Mg, Na, K and N were as follows: 3.1, 0.6, 5.0, 6.6 and 19.0 gm. and their retentions were 0.37, 0.11, 0.56, 1.40 and 3.06 gm., respectively. The ingestion of P, Cl and S amounted to 2.7, 7.7 and 1.5 gm. with resultant daily storage of 0.26, 0.60 and 0.34, respectively. Although the balances were continuous and the ingestion fairly constant the storage fluctuated markedly resulting at term in a large maternal reserve.

During lactation lowered rates of storage and even large losses were encountered as in previous short time balance studies.[1] The average daily balances for Ca, Mg, Na, K and N were — 0.48, — 0.03, + 0.47, + 0.87 and — 0.56 gm. and for P, Cl and S, — 0.37, + 0.30 and — 0.22, respectively

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[1] J. Biol. Chem., vol. 86, p. 37 (1930); ibid, p. 59; ibid, vol. 91, p. 675 (1931); ibid, vol. 99, p. 507 (1933).

Cataract in rats fed on galactose. Helen S. Mitchell, Division of Home Economics, Massachusetts State College, Amherst, Mass. Work done at Battle Creek College, Battle Creek, Mich.

A previous publication reported the occurrence of cataractous changes in the eyes of all rats fed on rations containing lactose as the chief source of carbohydrate. Negative results with other carbohydrates tested led to an investigation of galactose as the next logical step. This sugar was fed to young rats at 35 per cent and 25 per cent levels corresponding to the galactose available from the 70 per cent and 50 per cent lactose rations fed in previous experiments.

Four rats on the 35 per cent galactose ration developed mature bilateral cataract in 12, 14, 14 and 37 days, respectively (average 19 days), whereas those on the 25 per cent galactose ration were somewhat more delayed. The average time for the development of mature bilateral cataract in forty-nine rats fed the 70 per cent lactose ration was 10 weeks, approximately four times as long.

Galactosuria was more severe on galactose than on lactose rations. The increased calcium content of cataractous eyes was apparent in a shorter time. Galactose rations caused no diarrhea—a consistent finding with lactose feeding.

Rapid and complete absorption of galactose from the intestine, a slow glycogenesis and low kidney threshold reported by previous workers seem to be indicated.

Degenerative changes in the lens capsular membrane due to galactose injury, followed by infiltration of inorganic ions might be sufficient to cause the precipitation of proteins of the crystalline lens.

Role of the liver and pancreas in the dextrose tolerance curve. Samuel Soskin, The Metabolic Laboratory, Department of Physiology, Michael Reese Hospital, and Department of Physiology, The University of Chicago.

It has been shown that the presence of the pancreas is not essential to the normal dextrose tolerance curve, while the presence of the normal liver is essential. One of the major factors in determining the occurrence of the normal curve, is a homeostatic reaction of the liver, in which it decreases its supply of sugar to the blood in response to the influx of exogenous sugar.

These results and conclusions yield a new basis for interpreting those metabolic disturbances in which abnormal dextrose tolerance curves occur. The application of these facts to toxemia, diabetes, starvation, high carbohydrate therapy and 'insulin resistance' is briefly discussed.

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